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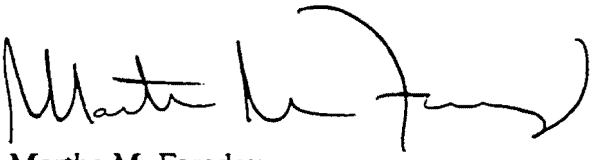

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ABSTRACT

Title of Thesis: Effects of Nicotine Administration and Stress on Sensory-Gating Depend on Rat Strain and Sex

Martha M. Faraday, Master of Science, 1998

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The present experiments investigated effects of nicotine administration, nicotine cessation, and stress (environmental and physical) on the acoustic startle reflex (ASR) and pre-pulse inhibition (PPI) of the ASR (measures of sensory-gating) in males and females of two rat strains. Experiment 1 examined effects of nicotine on 192 Long-Evans rats (a non-albino strain) in individual or crowded housing. For males, nicotine increased startle and PPI in the crowded condition but decreased these responses in the individually-housed condition. For females, nicotine reduced ASR and PPI regardless of housing condition. Experiment 2 examined effects of nicotine (0, 6, or 12 mg/kg/day) and immobilization (IM) stress on ASR and PPI of male and female Long-Evans and Sprague-Dawley (albino) rats (N = 240). Nicotine decreased ASR and PPI responses of Long-Evans subjects. Nicotine enhanced Sprague-Dawley subjects' responses. Stress increased responses of Sprague-Dawley males and Long-Evans females but decreased responses of Sprague-Dawley females and Long-Evans males.

Effects of Nicotine Administration and Stress

On Sensory-Gating

Depend on Rat Strain and Sex

by

Martha M. Faraday

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INTRODUCTION

Some people smoke; Others do not. Some individuals appear destined to become lifelong smokers after a single experience with tobacco, whereas others require multiple exposures. Still others -- so-called "chippers" -- are able to smoke or not smoke as they wish. Smokers also differ in amounts smoked, ranging from the person who smokes a few cigarettes a day to the chain-smoker who lights the next cigarette from the dying butt of the previous one. Success in quitting smoking depends as well on the individual. Some people are able to stop easily on their own; others require multiple attempts, pharmacologic support, and therapeutic interventions; and some individuals are never able to quit.

These differences in the initiation, maintenance, and cessation of smoking behavior have important implications in a society where cigarettes are widely available and easily obtainable. Cigarette-smoking is a costly major health hazard that contributes to 400,000 deaths each year in the U.S. (USDHHS, 1988; USPHS, 1992; Grunberg, Brown, & Klein, 1997). Despite the well-documented detrimental health effects of smoking, about 50 million Americans (one-quarter of the U.S. population) continue to smoke. The large individual differences in who smokes, in how much they smoke, and in ability to quit smoking suggest that individuals carry different vulnerabilities to become and remain smokers. One component of this individual vulnerability may be the specific effects of nicotine -- the primary, active, and addictive component of tobacco -- that the individual experiences. The present work examined one specific effect of nicotine (i.e., nicotine's effects on attention), with and without stress (an important variable that may

interact with nicotine) in an animal model that included several different genotypes (i.e., sex and strain). The present research examined effects of nicotine on attention because: **1)** reports suggest that nicotine affects attention but many of the studies lack methodological controls and rigor (e.g., Wesnes & Warburton, 1983; Heishman, Taylor, & Henningfield, 1994); **2)** it has been suggested that nicotine's effects on attention may be important to help explain smoking-stress interactions but this argument is based on limited data (Acri, 1994); and, **3)** the available, relevant literature has not systematically included gender or genotype. Background material pertinent to this work, including reported effects of nicotine, smoking and stress, smoking and individual differences, genotype and smoking, and clinical use of nicotine, is provided first. Then, a complete literature review of studies relevant to the present work is provided.

Major Effects of Nicotine that Contribute to Self-Administration

The Surgeon General has concluded that most moderate to heavy smokers smoke because they are addicted to nicotine (USDHHS, 1988). However, individuals also report that they smoke for additional reasons. Some individuals, often women, report that they use cigarette-smoking as a means of controlling body weight and suppressing appetite (Gritz, 1986; Grunberg, Winders, & Wewers, 1991; Klesges & Klesges, 1988; Klesges, Meyers, Klesges, & La Vasque, 1989). Other smokers indicate that smoking alleviates boredom (Parrott, 1995), reduces anxiety (Pomerleau, Turk, & Fertig, 1984; Gilbert, Robinson, Chamberlin, & Spielberger, 1989; Kassel & Shiffman, 1997), tempers hostility (Cherek, 1981; Cherek, Bennett, & Grabowski, 1991), and generally assists in mood regulation (Parrott, 1995). Further, some smokers report that smoking enhances

attentional processes and cognitive performance (Russell, Peto, & Patel, 1974; Wesnes & Warburton, 1983; USDHHS, 1988; Heishman et al., 1994). Coping with stress also is a reported reason for smoking, and is reflected in the fact that stress increases smoking rates and enhances the likelihood of relapse from smoking cessation (Shiffman, 1985; Wills & Shiffman, 1985; Shiffman, 1982; USDHHS, 1988).

Cigarette Smoking and Stress

The role that stress may play in maintenance of smoking behavior and in relapse from smoking cessation illustrates the complexity of nicotine's effects separate from its addictive properties. Although smokers report that cigarette-smoking is stress-relieving, biochemical and physiological indices of stress (e.g., stress hormones, heart rate, blood pressure) suggest that stress and nicotine have additive effects (MacDougall, Dembroski, Slaats, Herd, & Eliot, 1983; Perkins, Epstein, Jennings, & Stiller, 1986; Morse, 1989; Pomerleau & Pomerleau, 1990). That is, although the organism's peripheral biochemistry and physiology indicate a highly stressed state, humans in the laboratory and in epidemiological studies say that smoking a cigarette eases the psychological experience of stress. With regard to the stress-relapse relationship, it has been proposed that stress may induce relapse because physical manifestations of stress mimic sensations of nicotine withdrawal (Grunberg & Baum, 1985). However, relapse from smoking cessation does not appear to be attributable solely to the experience of withdrawal symptoms. For example, Shiffman (1982) reported that approximately half of smokers relapsing from cessation cited affect regulation rather than withdrawal symptoms *per se* as the reason for returning to smoking. Taken together, the variability in reasons for smoking and in

reasons for relapsing from cessation indicate that individuals smoke for many reasons in addition to nicotine addiction.

Smoking and Individual Differences

It is possible, therefore, that in addition to its addictive properties, the particular effects of nicotine experienced may play a role in the initiation, maintenance, and cessation of smoking behavior. The individual, for example, who experiences nausea and agitation when smoking a first cigarette may be less likely to become a habitual smoker than an individual who finds the experience pleasant or neutral (Silverstein, Feld, & Kozlowski, 1980; Pomerleau, 1995). Similarly, a person who experiences cognitive sharpening or stress relief from cigarette smoking may be more likely to become a heavy smoker and find eventual cessation difficult than an individual who experiences only mild weight reduction. The importance of considering individual differences in reasons for smoking as part of effective cessation has been highlighted by advocates of the custom-tailored approach to cessation (Grunberg, 1995).

All of the individual differences relevant to smoking -- in reported effects of smoking as well as in who smokes, in how much individuals smoke, and in ability to quit smoking -- may reflect differences in psychological, environmental, or biological variables. In particular, the extent to which these individual differences are biologically-based is not clear. The fact that all smokers do not have the same experience when smoking could partly be explained by peripheral and/or central nervous system differences. For example, people may experience different effects of nicotine as a result of different rates of nicotine metabolism or different central nicotinic cholinergic receptor

distributions or sensitivities. In other words, the genotype of smokers may be relevant to smoking behavior.

Genotype and Smoking

Genotype is a biological, "hard-wired" individual difference that includes ethnicity in humans or strain in rats of subjects as well as gender or sex of subjects. Individuals and animals of different genotypes may manifest differential central and peripheral physiological and biochemical responses and different behaviors. In humans, for example, ethnically-derived differences in liver enzymology mediate differential drug sensitivities (Chien, 1993; Matthews, 1995). Human sex differences also exist in the effects of drugs such as cocaine (Lukas, Sholar, Fortin, Wines, & Mendelson, in press) and nicotine (Perkins, 1996). In animals, strain differences in drug responses (Marks, Stitzel, & Collins, 1989; Rigdon, 1990; Brown, 1997) and sex differences in drug responses (Grunberg et al., 1991, Lex, 1991; Mendelson & Mello, 1986) and stress responses (Brown & Grunberg, 1995; Faraday, Scheufele, Rahman, & Grunberg, 1997; Klein, 1997) have been documented.

Twin studies indicate that smoking behavior is, at least in part, genetically-controlled. Specifically, there is a mean heritability estimate of 53% for tobacco use (Hughes, 1986). In fact, genetic factors appear to contribute to several aspects of smoking behavior, including initiation, age of onset, and number of cigarettes smoked per day (Heath & Martin, 1993; Eaves & Eysenck, 1980; Hannah, Hopper, & Mathews, 1984). In addition, smoking prevalence data reveal striking differences among ethnic groups (SAMHSA, 1996a). These differences are likely the result of many influences,

including environmental as well as psychological factors. These data also are consistent, however, with the idea that genotype, including sex, may play a role in smoking behavior. For example, in 1995 22.9% of whites aged 12-17 were current smokers while only 11.8% of African-Americans and 15.5% of Hispanics reported current cigarette use. In the 18-25 year old age group, 38.6% of whites were current smokers as compared to 24.1% of African-Americans and 28.0% of Hispanics. In the 26-34 age group, dissimilar patterns remained with 37.3% of whites reporting current smoking but 34.4% of African-Americans and only 26.7% of Hispanics using cigarettes currently. Detailed analyses of 1994 data, which revealed similar patterns among ethnic groups, indicated that whites reported greater lifetime use (78%) of cigarettes than African-Americans (62%) and Hispanics (61%). Whites also had more than double the proportion of heavy smokers (defined as smoking a pack or more a day) (15%) than African-Americans (7%) and three times the proportion of heavy smokers as Hispanics (5%) (SAMHSA, 1996b).

When data are examined within each ethnic group by sex, important differences also emerge. Data from 1995 indicated that although smoking rates among white males and females (collapsed across age group) were similar (30.9% and 28.5% respectively), African-American males smoked at higher rates (31.0%) than African-American females (25.8%), and Hispanic males smoked at much higher rates (31.4%) than Hispanic females (18.0%) (SAMHSA, 1996a). SAMSHA analyses (based on 1994 data) indicated that among 18-25 year olds, significantly more white (39.1%) and Hispanic males (37.0%) reported current smoking than African-American males (28.1%) and white females (38.0%) had higher smoking rates than Hispanic (18.0%) or African-American females

(22.2%). In the older age groups (26-34, and 35+), ethnic differences among males disappeared but white (28.3%) and black females (29.4%) remained more likely to report current cigarette use than Hispanic females (21.1%). Among adults, Hispanic females had the lowest prevalence of lifetime, past-year, and current cigarette use of all ethnic-gender groups.

With regard to gender, it also is noteworthy that smoking rates among adult women (collapsed across ethnicity) have risen over the last 40 years and rates of smoking among adolescent boys are being overtaken by adolescent girls' smoking rates (Gritz, 1986; USDHHS, 1989, 1994). These trends have been paralleled by increasing availability of low nicotine-content cigarettes (Gritz, 1986; USDHHS, 1989). One interpretation of the concurrent increases in female smoking rates and low-nicotine cigarette production is that greater availability of low nicotine cigarettes decreased the likelihood that females, more sensitive to many nicotine effects than males, would have a negative first experience with cigarettes (Silverstein et al., 1980). The rise in female smoking rates, therefore, also has been interpreted as evidence of greater female sensitivity to nicotine's effects consistent with laboratory studies (Tepper, Wilson, & Schlesinger, 1979; Bättig, Buzzi, & Nil, 1982; Silverstein et al., 1980; Levin, Morgan, Galvez, & Ellison, 1987; Winders & Grunberg, 1989; Grunberg et al., 1991; Perkins, 1996).

Whether prevalence differences in smoking among ethnic groups, between sexes, or between sexes within ethnic groups are the result of environmental, psychological, or biological factors is not known. Understanding the role of biologically-based individual

differences in nicotine's effects, however, may contribute to knowledge about the mechanisms of nicotine's actions as well as to optimization of cessation and prevention strategies.

Relevance to Clinical Use of Nicotine

Biologically-based individual differences in nicotine's effects also may be relevant in other clinical contexts. Specifically, some effects of nicotine may have potential therapeutic value. The reported effects of smoking to enhance cognitive processes, for example, have resulted in use of nicotine as an experimental treatment for disorders characterized by impaired thought processes such as Alzheimer's disease (Newhouse, Sunderland, Tariot, Blumhardt, Weingartner, et al., 1988; Sahakian & Jones, 1991; Jones, Sahakian, Levy, Warburton, & Gray, 1992; Levin, Karan, & Rosecrans, 1993; Levin & Rosecrans, 1994). It is possible that biologically-based individual differences, such as genotype and gender, determine to some extent whether or not this effect of nicotine is experienced. The fact that some, but not all, smokers report this effect is consistent with this hypothesis. Establishing whether biological factors contribute to individual differences in nicotine-induced cognitive enhancement, therefore, is important to determine whether, how, and for whom nicotine or its analogs might best be used therapeutically.

In summary, it is well-known that there are individual differences in who smokes, in amounts smoked, in difficulty quitting, and in reported effects of smoking. It is not known to what extent these individual differences are the product of psychological, environmental, or biological influences. The human and animal literatures suggest that

the smoker's genotype and sex -- biologically-based individual differences -- may partly explain why some but not all individuals smoke and why different people report different effects of smoking. This information is of potential importance to prevent smoking initiation, to maximize cessation success, and also to possibly use nicotine and similar substances as therapeutic agents.

The Present Research: Attentional Effects of Nicotine and Stress

The present research examined the effects of nicotine and stress on attention for three reasons. First, there is a substantial literature examining effects of drugs on attentional indices in humans and rats. Second, the human smoking literature indicates that some but not all smokers experience attentional enhancement when smoking. These effects, therefore, may occur along a continuum and may be meaningfully different across strains and sexes of rats. Third, in humans and rats nicotine attenuates the psychological and behavioral experience of stress but paradoxically exerts biochemical and physiological effects that are additive with effects of stress. The mechanism by which nicotine normalizes behavior and psychological experience under stress is unknown. It is possible, however, that nicotine-induced attentional enhancement may contribute to stress reduction. That is, the stressed rat administered nicotine may experience normal attentional processes and therefore respond to stimuli in a stressful environment normally. Similarly, the stressed smoker smoking may preserve attentional focus despite a challenging environment and thus cope more effectively. The extent to which these nicotine effects depend on subjects' sex and genotype is relevant to understanding smoking behavior, to possible clinical use of nicotine, and to the broad and complex

stress literature.

The experiments reported in this thesis were conducted with rats as subjects. Use of a rat model to investigate the role of genotype and sex in attentional effects of nicotine, of stress, and of nicotine and stress together allowed experimental control of potentially confounding variables that are difficult to eliminate in human studies. For example, rat subjects' environment (e.g., light-dark cycle, temperature, humidity, food, water) was controlled 24-hours-a-day for the duration of the studies. This animal model also is an appropriate paradigm to examine questions of possible genotypic and sex differences in attentional effects of nicotine, stress, and nicotine and stress because of the extensive and relevant animal and human literatures. The necessary conceptual underpinnings are reviewed below and include: **1)** that nicotine is the agent in tobacco responsible for the reported effects of smoking and that these effects have been demonstrated in humans and rats; **2)** that a behavioral paradigm exists in humans and rats that indexes attentional processes; **3)** that responses in this paradigm are affected by drugs including nicotine, and by individual differences in drug responses; **4)** that stress alters responses of humans and rats, and alters effects of drugs; **5)** that responses in this paradigm are affected by stress, and by stress and nicotine together; and, **6)** that the paradigm is sensitive to the individual differences of genotype and gender or sex in humans and rats.

1) Effects of Nicotine

Nicotine is the primary, active and addictive pharmacologic agent in tobacco (USDHHS, 1988; West & Grunberg, 1991). In addition to its addictive properties, it is well-established that nicotine has other actions that contribute to its use. In fact, literature

relevant to biologically-mediated individual differences in nicotine's effects indicates that nicotine is the agent responsible for the major reported effects of smoking. Specifically, in empirical studies, nicotine administration decreased body weight in humans and rats (Grunberg, 1982; Winders & Grunberg, 1989), decreased aggression in humans (Cherek, 1981; Cherek et al., 1991) and in rats (Silverman, 1971; Scheufele, 1997), and altered biochemical and behavioral responses to stress in rats (Benwell & Balfour, 1982; Cam & Bassett, 1983; 1984; Sharp, Beyer, Levine, Morley, & McAllen, 1987; Peck, Dilsaver, & McGee, 1991; Acri, 1992, 1994; Takada, Ihara, Urano, & Takada, 1995) and in humans (MacDougall, Musante, Castillo, & Acevedo, 1988; Gilbert, Robinson, Chamberlin, & Spielberger, 1989; Pomerleau & Pomerleau, 1990; Levin, Rose, Behm, & Caskey, 1991; Smits, Temme, & Thien, 1993).

Nicotine administration in laboratory settings also has been reported to affect cognitive processes. Whether nicotine improves, impairs, or has no effect on cognitive task performance depends on many factors, including the nature of the subject pool and the task involved. With regard to attention-related tasks, nicotine administration has been reported to enhance performance on a vigilance task in smokers (Wesnes & Warburton, 1983; Wesnes, Warburton, & Matz, 1983; Gilbert, Estes, & Welser, 1997), in non-smokers (Wesnes & Warburton, 1984, Wesnes & Revell, 1984; Provost & Woodward, 1991), in Alzheimer's patients (Newhouse et al., 1988; Sahakian & Jones, 1991; Jones et al., 1992; Levin et al., 1993; Levin & Rosecrans, 1994), and in ADHD patients (Levin et al., 1993; Levin et al., 1995; Levin & Rosecrans, 1994). The strength of these findings in smokers and non-smokers, however, has been challenged. Specifically, methodological

problems such as small sample size, lack of placebo controls and single- or double-blind procedures, use of nicotine delivery systems with significant inter-subject variability (e.g., cigarette-smoking), and the practice of using as subjects smokers who were deprived for varying amounts of time before testing complicate interpretation of these results (Heishman et al., 1994). When only studies using rigorous experimental methodology are considered, nicotine's performance-enhancing effects in deprived smokers appear to generally reverse effects of withdrawal and return subjects to baseline (Heishman et al., 1994). In non-deprived smokers and nonsmokers, nicotine's effects on cognitive performance are subtle and task-specific. Specifically, in non-deprived smokers nicotine administration reduced reaction time on a choice reaction time task and decreased errors on a tracking task (Hindmarch, Kerr, & Sherwood, 1990; Kerr, Sherwood, & Hindmarch, 1991). In nonsmokers nicotine administration resulted in faster Stroop responses (Provost & Woodward, 1991), attenuated vigilance performance decrements (Wesnes et al., 1983), improved choice reaction time (Le Houezec et al., 1994), and improved rapid visual information-processing (Foulds et al., 1996). In an experimental series that systematically varied task requirements, Spilich et al. (1992) concluded that cigarette-smoking can have positive effects upon the performance of simple, repetitive tasks but negative effects on high-demand tasks that involve working and long-term memory.

Whether these varying reports of nicotine's effects also depend on the gender and genotype of subjects employed is not clear. The studies conducted in non-clinical populations included subjects predominantly of one ethnicity (Western European Caucasian) and usually tested only male subjects. In studies with male and female

subjects (e.g., Wesnes et al., 1983; Provost & Woodward, 1991; Spilich et al., 1992) the sparse demographic information reported suggests limited ethnic sampling and analyses by gender or gender differences are generally not reported. It is impossible to tell, therefore, whether or not the investigators looked for differences. In any case, it is possible that gender differences were not detected because the number of male and female subjects per cell was too few (typically n = 6 of each sex) to reliably distinguish sex differences in these responses. In addition, recent studies indicate that men and women differ in baseline information processing abilities (Swerdlow et al., 1993) and that in women these processes vary with the menstrual cycle (Broverman et al., 1981; Swerdlow et al., 1997), further complicating interpretation of studies with human females as subjects. With six women per cell, variability in menstrual cycle phase might obscure effects of nicotine on attention. In addition, work on other sympathomimetics (e.g., caffeine) indicates that effects of drugs in this class also can depend on menstrual cycle phase (Erickson et al., 1985; Arnold, Petros, Beckwith, Coons, & Gorman, 1987). The effects of nicotine on attentional processes in humans, therefore, have not been established across genotypes and genders.

2) The Acoustic Startle Reflex and Pre-Pulse Inhibition: Behavioral Indices of Attention

Changes in attentional processes as a result of nicotine administration also have been reported in rats as measured by the acoustic startle reflex (ASR) and pre-pulse inhibition paradigm (Acri, Morse, & Grunberg, 1991; Acri, 1992; Helton, Modlin, Tizzano, & Rasmussen, 1993; Acri, Morse, Popke, & Grunberg, 1994; Acri, 1994;

Popke, Acri, & Grunberg, 1994; Curzon, Kim, & Decker, 1994; Acri, Brown, Saah, & Grunberg, 1995; Rasmussen, Czachura, Kallman, & Helton, 1996). The ASR and pre-pulse inhibition of the ASR are behavioral responses believed to index central processes related to information processing (Swerdlow, Caine, Braff, & Geyer, 1992) and possibly attention (Acri et al., 1991; Acri, 1992, 1994; Acri et al., 1994; Grunberg, Acri, & Popke, 1994; Popke et al., 1994; Acri et al., 1995). The acoustic startle reflex is a characteristic sequence of involuntary, muscular responses elicited by a sudden, intense acoustic stimulus (Davis, 1984). Jumping in response to an unexpected car backfire is an everyday example of the startle reflex. The reflex is present in all mammals, including humans and rats, and is considered an index of reactivity to external acoustic stimuli. In addition, because the reflex can be elicited using the same stimuli across species (Swerdlow, Braff. Taaid, & Geyer, 1994), the paradigm has face validity for generalizing from an animal model to human issues.

Pre-pulse inhibition (PPI) of the acoustic startle reflex (ASR) occurs when the startling stimulus is preceded by a non-startling acoustic stimulus by a short interval (about 100 msec). The presence of the pre-pulse results in measurably reduced startle amplitude (Graham, 1975; Braff et al., 1978). In the everyday example of a car backfire, the ability of this loud noise to startle would be reduced if the listener also heard the engine sounds immediately preceding the backfire. This reduction in startle amplitude is pre-pulse inhibition of the ASR. As with the ASR, the phenomenon of pre-pulse inhibition occurs in humans and in rats. Pre-pulse inhibition is believed to index an innate sensory-cognitive-motor "gating" mechanism that operates at a non-volitional level

and underlies the organism's ability to select relevant stimuli from the environment while screening out irrelevant information (Swerdlow, Caine, Braff, & Geyer, 1992). PPI also has been interpreted to reflect processes associated with attention (Acri et al., 1991; Acri, 1992, 1994; Acri et al., 1994; Grunberg et al., 1994; Popke et al., 1994), and in humans PPI is negatively correlated with distractibility (Karper et al., 1996). Because the ASR-PPI paradigm indexes substrates believed to underlie information-processing and attention, use of this procedure avoids the complications of task choice.

3) <u>ASR-PPI, Drug Effects, and Individual Differences</u>

Human Studies. The acoustic startle reflex and pre-pulse inhibition are mediated by different brain pathways and can be separately manipulated by drugs or by environmental conditions. For example, in normal human volunteers caffeine (Schicatano & Blumenthal, 1995) and yohimbine (Morgan et al., 1993) increased startle amplitudes, ethanol reduced startle and eliminated PPI (Grillon, Sinha, and O'Malley, 1994), diazepam blocked startle potentiated by fear (Patrick, Berthot, & Moore, 1996), and pleasant smells decreased startle while unpleasant smells increased startle (Miltner, Matjak, Braun, Diekmann, & Brody, 1994). One study has examined the effects of smoking on ASR and PPI. Specifically, Kumari and colleagues (1996) reported that cigarette-smoking decreased startle amplitudes and increased PPI in a group of adult male smokers who had been deprived of cigarettes overnight.

In human clinical populations disrupted PPI is manifested in disorders where the ability to gate unwanted sensory information, cognitions, or motor movements is impaired. For example, individuals with Attention Deficit Hyperactivity Disorder

(ADHD) have difficulty attending to appropriate stimuli in the environment (Levin et al., 1995), schizophrenic patients "hear" internal cognitions and sensations as real, external voices, and Huntington's disease sufferers struggle to suppress unwanted motor movements (Swerdlow et al., 1992). Whereas startle behaviors frequently remain intact in these disorders, all of these conditions are characterized by impaired pre-pulse inhibition (Geyer & Braff, 1987; Swerdlow et al., 1992). In general, drugs that have anti-psychotic action in humans are reported to normalize pre-pulse inhibition in these individuals and in animal subjects with pharmacologically-induced PPI disruption (Swerdlow et al., 1992).

Rat Studies. In rats startle and PPI also can be manipulated by drugs. For example, startle amplitude has been reported to increase in response to the dopamine agonists apomorphine (Davis, 1988), d-amphetamine (Davis, Svensson, Aghajanian, 1975; Kokkinidis & Anisman, 1978; Davis, 1988), and cocaine (Harty & Davis, 1985), and decrease in response to ethanol (Pohorecky, Cagan, Brick, & Jaffe, 1976) and haloperidol (Mansbach, Geyer, & Braff, 1988). In contrast to ASR-enhancing effects of dopamine agonists, pre-pulse inhibition is increased by dopamine antagonists and reduced by dopamine agonists. Specifically, increased PPI has been found after treatment with haloperidol, raclopride, and buspirone (antagonists at the D2 receptor) (Johansson, Jackson, Zhang, & Svensson, 1995) and reduced PPI has been found in response to treatment with apomorphine (Swerdlow, Vaccarino, Amalric, & Koob, 1986), d-amphetamine (Mansbach et al., 1988; Swerdlow et al., 1990), and cocaine (Swerdlow et al., 1992). PPI responses also are reduced by compounds related to methamphetamine

such as MDMA (3,4-methylenedioxymethamphetamine) (Mansbach, Braff, & Geyer, 1989) and by lysergic acid-diethylamide (LSD) (Geyer & Braff, 1990).

Effects of nicotine and nicotine cessation on ASR and PPI in rats also have been studied. This literature, however, is contradictory. Nicotine has been reported to enhance startle amplitude and pre-pulse inhibition (Acri et al., 1991; Acri, 1992, 1994; Acri et al., 1994; Popke et al., 1994; Acri et al., 1995) and also to have no effect on startle but enhance pre-pulse inhibition (Curzon et al., 1994). Nicotine cessation has been reported to have no effect on startle and reduce pre-pulse inhibition (Acri et al., 1991; Acri, 1992) and also to enhance startle (Helton et al., 1993; Rasmussen et al., 1996).

These inconsistent results might be explained by two factors: the strain of subjects used and methodological differences among studies. It is noteworthy that studies reporting enhancement of ASR and PPI during nicotine administration used albino rats of the Sprague-Dawley strain as subjects (Acri et al., 1991; Acri, 1992, 1994; Acri et al., 1994; Popke et al., 1994; Acri et al., 1995). Studies reporting no effect on startle and reduced PPI during nicotine cessation (Acri et al., 1991; Acri, 1992) also used Sprague-Dawley rats. In studies reporting no effects of nicotine on startle and enhancement of PPI only during nicotine administration (Curzon et al., 1994), a non-albino strain -- Long-Evans hooded rats -- was used. Long-Evans subjects also were used in studies reporting enhancement of startle in cessation only (Helton et al., 1993; Rasmussen et al., 1996).

Important differences among studies in addition to strain of subject, however, also exist. The studies reported above all used male subjects except for Acri et al. (1994) and Popke et al. (1994) which used female Sprague-Dawley rats. Some studies used a chronic

nicotine administration paradigm via minipump (Acri et al., 1991; Acri, 1992, 1994; Acri et al., 1995; Helton et al., 1993; Rasmussen et al., 1996). Other studies used acute nicotine injections (Acri et al., 1994; Popke et al., 1994; Curzon et al., 1994). In addition, the only studies that examined female rats also used acute nicotine injections (Acri et al., 1994; Popke et al., 1994).

Further, the time of ASR and PPI testing during the circadian cycle also varied across studies. Some studies tested responses during the active portion of the cycle (dark portion) (Acri et al., 1991; Acri, 1994; Acri et al., 1994) and other studies tested responses during the resting-feeding portion of the cycle (light portion) (Acri et al., 1995; Curzon et al., 1994). Some studies do not report time of testing (Helton et al., 1993; Popke et al., 1994; Rasmussen et al., 1996).

Time of testing is relevant because startle amplitudes are greater during the dark cycle (Chabot & Taylor, 1992). Specifically, startle amplitudes increase by up to 100% during the dark portion of the daily cycle over mean startle values measured during the light portion. Because PPI is calculated as a portion or percentage of startle amplitude, time of testing may affect findings with regard to this measure. Testing during the light portion of the day when startle responses are minimal may obscure drug effects on startle and may not reflect meaningful changes in PPI. That is, when baseline startle amplitude is low, small but consistent startle reductions (on the order of a few grams) produced by a pre-pulse may reach statistical significance but lack practical, clinical significance. In addition, data indicating that nicotine administration enhances responses of an animal tested during a period when it normally would be asleep cannot be extrapolated to mean

that nicotine also will enhance the responses of an animal that is already awake and active. This information has clinical implications for the use of nicotine or nicotine analogs to improve attentional processes in disease states; i.e., substances relevant to clinical use must have effects robust enough to bolster cognitive functioning while the patient is awake and alert.

Additional methodological differences among studies complicate interpretation. For example, the form of nicotine used varied across studies. Some experiments used nicotine dihydrochloride (Acri et al., 1991; Acri, 1992, 1994; Popke et al., 1994; Acri et al., 1994; Acri et al., 1995), some used nicotine tartrate (Helton et al., 1993), some used nicotine bitartrate (Curzon et al., 1994) and some experiments employed nicotine ditartrate (Rasmussen et al., 1996).

The differences in solubility among nicotine forms also require minipumps of different sizes for delivery of reported dosages. Specifically, the one-milliliter capacity Alzet Model 2002 was used in studies employing nicotine dihydrochloride but the larger, two-milliliter Model 2ML2 minipump was used in studies employing nicotine tartrate and nicotine ditartrate. Although in theory subjects received the reported dosage of nicotine as long as the correct size minipump was filled with the correct solution, in one study (Helton et al., 1993) subjects did not lose weight as a result of nicotine administration (administered as nicotine ditartrate) or experience rebound weight gain in cessation despite the fact that the dosage of nicotine used (6 mg/kg/day) produced body weight effects in other studies (e.g., Winders & Grunberg, 1989). Other studies do not report body weight data (Rasmussen et al., 1996) so it is not possible to determine if subjects

received appropriate drug amounts. In any case, it cannot be ruled out that these procedural differences also might account for some or all of the observed behavioral differences between strains because the small minipump was used in studies of Sprague-Dawley subjects whereas the larger pump was used in studies of Long-Evans subjects.

It is not clear, then, to what extent the ASR and PPI differences reported constitute a strain difference and to what extent they are accounted for by the methodological dissimilarities reviewed above. Work on other drugs indicates that strain of subject can affect ASR and PPI responses to drugs. For example, Swerdlow and colleagues (1992) noted that apomorphine disrupted PPI in rats of the Wistar strain but not in Sprague-Dawley subjects and Rigdon (1990) reported that the same drug had no effect on Wistar subjects' startle but increased Sprague-Dawley subjects' startle. With regard to nicotine, Collins and colleagues indicated in mice (Marks, Burch, & Collins, 1983; Marks, Romm, Gaffney, & Collins, 1986; Collins, Miner, & Marks, 1988; Marks et al., 1989; Pauly, Ullman, & Collins, 1990; Grun, Pauly, Bullock, & Collins, 1995) that, depending on the strain of the subjects, nicotine enhanced acoustic startle responses, decreased startle responses or had no effect on startle responses.

Determining the existence of a true strain difference between Sprague-Dawley and Long-Evans rats has important implications for clinically relevant work on effects of nicotine. Some investigators (Acri et al., 1991; Acri, 1992, 1994; Acri et al., 1994; Grunberg et al., 1994; Popke et al., 1994; Acri et al., 1995) have interpreted enhancement of startle and PPI in Sprague-Dawley subjects as nicotine increasing attentiveness to salient stimuli and enhancing sensory-gating and attention, analogous to the attentional

enhancement reported by some human smokers when they smoke. It cannot be concluded from these studies, however, that nicotine's effects to enhance attention generalize across rat strains and, by extension, across different human genotypes.

In addition, genotype can be broadly construed to include another biological variable relevant to drug effects -- i.e., the sex of subjects. For example, in studies examining sex differences in opioid self-administration, female rats consumed more fentanyl than did male rats but male rats displayed more withdrawal symptoms (Klein, Popke, & Grunberg. 1994; Brown, Klein, Rahman, & Grunberg, 1995). Human females are less sensitive to cocaine's effects than males (Lukas et al., in press). With regard to nicotine's effects on body weight, female humans and rats have been found more sensitive than males, exhibiting greater body weight reductions when exposed to nicotine and greater body weight gains when abstaining from nicotine (Grunberg, 1982; Grunberg, Bowen, & Winders, 1986; Levin et al., 1987; Grunberg et al., 1991).

It is important to note that all of the ASR-PPI studies cited in this discussion that used a chronic administration paradigm also used male rats as subjects. In studies using female Sprague-Dawley subjects (Acri et al., 1994; Popke et al., 1994) low doses of acutely administered nicotine enhanced ASR and PPI and high doses decreased these responses. Popke et al. (1994) reported that the magnitude of these effects was greater in females than in males. This pattern, therefore, also is consistent with females being more sensitive to nicotine's effects than males (i.e., the female dose-response curve is shifted to the left of the male dose-response curve). Whether sex differences exist in Sprague-Dawley responses to chronic nicotine administration, however, is not known.

Additionally, whether sex differences exist in non-albino strains in these drug effects is not known. The extent to which the sex of subject matters in nicotine's attentional effects, however, is clearly relevant to establishing the role of biologically-based individual differences in these and other effects of nicotine.

4) Stress and Drug Effects

The responses of male and female humans and rats also can be altered by exposure to environmental conditions that result in the experience of stress. Stress consists of a stressor (the environmental condition or event), stress responses, and factors that might mediate the effects of stress on the organism (Glass & Singer, 1972; Baum, Grunberg, & Singer, 1982; Cohen, Evans, Stokols, & Krantz, 1986; Grunberg & Singer, 1990). The experience of stress can be indexed in several ways, including behavioral and peripheral biochemical changes. In humans, stress alters feeding behaviors (Grunberg & Straub, 1992; Greeno & Wing, 1994; Klein, Faraday, & Grunberg, 1996), impairs cognitive performance, decreases persistence on frustrating tasks, and decreases attention (Glass & Singer, 1972; Cohen et al., 1986; Baum, Davidson, Singer, & Street, 1987; Baum, 1990). Stress also increases cigarette-smoking and other drug use and increases relapse rates from smoking cessation (Shiffman, 1982; 1985; Wills & Shiffman, 1985; USDHHS, 1988).

In rats, a variety of environmental manipulations have been used to produce biochemical stress responses and stress-induced behavioral alterations. These procedures include short periods of physical immobilization as well as the use of different housing conditions (individual housing vs. crowded housing). Immobilization is a non-painful,

physical stressor in which the subject is held for a brief period of time (typically 20 minutes once a day) in a device that prevents movement. This procedure has produced reliable peripheral biochemical changes in the form of elevated adrenocorticotropin hormone (ACTH), beta-endorphins, and corticosterone consistent with a stress response (Kant et al., 1983; Flores, Hernandez, Hargreaves, & Bayer, 1990; Acri, 1992; 1994; Raygada, Shaham, Nespor, Kant, & Grunberg, 1992; Klein, 1997). These responses do not diminish with repeated exposure to the stressor and are similar in males and females (Kant et al., 1983).

In contrast to the lack of sex differences in biochemical responses to immobilization, powerful sex differences have been reported in response to housing conditions. Specifically, Brown and Grunberg (1995) found that crowded housing conditions stressed male rats but calmed female rats as indexed by peripheral corticosterone levels. This paradigm also has revealed that stress can alter the effects of drugs. Specifically, when male and female Wistar (an albino strain) subjects were housed either individually or in crowded groups, crowded (non-stressed) females self-administered more fentanyl (an opioid 100 times more potent that morphine) than did individually-housed (stressed) females and no differences in drug consumption by males were found (Brown et al., 1995). These findings suggest that Wistar males and females are differentially sensitive to housing conditions as indexed by corticosterone levels as well as by drug self-administration responses. Whether males and females of other strains (e.g., Sprague-Dawley, Long-Evans) also are differentially sensitive to housing conditions is not known. The effects of housing condition on responses to nicotine

administration and possible sex differences in these effects, however, are not known in albino or non-albino strains. Assessment of these responses across strains and sexes might shed further light on the role of individual differences in nicotine's effects.

5) ASR-PPI, Stress, and Nicotine

A number of studies have indicated that stress affects the acoustic startle reflex and pre-pulse inhibition. Whether stress enhances, has no effect, or diminishes these responses when compared to non-stress controls depends on a number of factors, including the particular stressor used. Specifically, investigators have reported that startle amplitudes are increased by foot shock (Warren, Pilcher, & Coopersmith, 1984), conditioned fear (Hijzen, Woudenberg, & Slangen, 1990), immobilization (Acri, 1992; 1994). and administration of the stress hormone corticotropin-releasing factor (CRF) (Swerdlow, Geyer, Vale, & Koob, 1986), but decreased by tail shock (Servatius, Ottenweller, Bergen, Soldan, & Natelson, 1994) and tail pinch (Sorenson & Swerdlow, 1982). Cold and warm forced swimming have been reported to have no effect on startle amplitudes (Leitner, 1989). With regard to PPI, investigators have reported that cold and warm swim stress diminished pre-pulse inhibition (Leitner, 1989) and foot shock had no effect on PPI (Warren et al., 1984). Effects of immobilization on PPI depended on subject's sex. Immobilization increased male Sprague-Dawley PPI (Acri 1992, 1994) but decreased female Sprague-Dawley PPI (Popke et al., 1994). Because most of the subjects in these studies were male albino rats of either the Sprague-Dawley or Wistar strains, it is not clear whether these ASR-PPI responses to stress generalize reliably to males and females of non-albino strains. Further, the effect of differential housing conditions on

ASR-PPI responses is not known.

Only one study has examined the interaction of chronic nicotine administration and stress on ASR-PPI responses. Acri (1992, 1994) reported that the effects of immobilization stress on ASR and PPI depended on nicotine dose. Specifically, Acri (1992, 1994) found that administration of 6 mg/kg/day nicotine to male Sprague-Dawley subjects, who also were exposed to immobilization stress, resulted in nicotine and stress having additive, enhancing effects on ASR and PPI. Administration of 12 mg/kg/day nicotine in stressed subjects, however, resulted in ASR and PPI responses that were indistinguishable from non-stressed saline control responses (Acri, 1992, 1994). The fact that high doses of nicotine when combined with stress produced behavioral responses similar to non-stressed, non-drug control subjects is consistent with the report of human smokers that cigarette-smoking alleviates stress. Whether chronic nicotine administration and stress interact similarly for females and for subjects of other strains is not known.

6) <u>ASR-PPI, Genotype, and Gender</u>

An extensive literature search indicated that to-date no studies have examined the role of genotype or ethnicity in human ASR and PPI responses. Human sex differences in startle responses and pre-pulse inhibition, however, have been investigated. Specifically, men and women have been reported to exhibit similar startle amplitudes but men have been found to exhibit greater PPI than women (Swerdlow et al., 1993). In addition, the pre-pulse inhibition of women varied with phase of the menstrual cycle. Specifically, Swerdlow and colleagues (1997) found that PPI was significantly reduced in the luteal vs. follicular phases of the menstrual cycle, with the most marked PPI reductions occurring

during the midluteal phase characterized by elevated estrogen and progesterone.

In rats, baseline genotypic differences in startle and PPI responses exist that depend on strain. Acri et al. (1995) found that Wistar subjects startled more to a given stimulus than did Sprague-Dawley subjects, and Sprague-Dawley subjects startled more than did Long-Evans subjects. Pre-pulse inhibition followed a similar pattern, with Wistars having greater PPI than Sprague-Dawleys, and Sprague-Dawleys exhibiting more PPI than Long-Evans animals. The subjects in this study, however, were males. Whether female responses vary across strains is not known. One study has examined sex differences in ASR-PPI responses: Swerdlow and colleagues (1997) reported that Sprague-Dawley males and females did not differ on startle amplitude or PPI. Whether sex differences in ASR and/or PPI exist in other strains is not known.

Summary and General Purposes of Studies

This review indicates that genotype, including sex of subject, may play an important role in the effects of nicotine on attentional processes and the effects of stress on these processes. In addition, it is possible that the interaction of nicotine's effects on attention with stress also may depend on the strain and sex of subjects. The responses of albino strain female subjects and non-albino strain male and female subjects to these manipulations are largely unknown. Altered behavioral responses reported in nicotine-treated animal subjects as a result of genotype and/or exposure to different stressors may reflect some of the individual differences reported by human smokers in effects of nicotine. Clearer understanding of the role of biological variables such as genotype, environmental variables such as stress, and the interaction of biological influences with

environmental conditions may yield clinically-relevant insights into smoking behavior and into clinical applications of nicotine and nicotine analogs.

This Master's project included two laboratory experiments. The general purposes of Experiments 1 and 2 were to investigate the contribution of individual differences to nicotine's attentional effects in a behavioral paradigm with cross-species generalizability: the acoustic startle reflex and pre-pulse inhibition. Specifically, the roles of strain, sex, and environmental conditions were chosen for examination because little is known about strain and sex differences in response to nicotine using this paradigm and little is known about the interactions of strain and sex with specific stressors. Two outbred rat strains were chosen as subjects -- Sprague-Dawleys and Long-Evans -- because these subjects are commonly used in experimental work and because findings about the ASR and PPI responses of these different subjects are inconsistent. In addition, outbred rat strains are bred for maximum variability. That is, there is substantial genetic variance among individuals of each strain. Use of outbred subjects, therefore, is appropriate when the goal is to examine differences that may extrapolate to human individual differences in a genotypically variable population. This information obtained in an animal model may be useful to understand how people of different genotypes and different sexes respond to nicotine and to various stressors, and how nicotine and stress interact for these individuals. This information also may be relevant to the question of why individuals are so variable in their likelihood of becoming a smoker, in their pattern of tobacco use, and in their ability to quit smoking.

Specific Purposes of Experiment 1 and 2

The specific purposes of Experiment 1 were to determine whether effects of nicotine to reduce body weight and to enhance ASR and PPI would generalize to a non-albino rat strain -- the Long-Evans strain. In addition, Experiment 1 was designed to examine effects of housing on ASR and PPI responses in these subjects, and to determine whether and how nicotine administration and housing, and nicotine cessation and housing, would interact to affect ASR and PPI responses. The purposes of Experiment 2 flowed from the results of Experiment 1. Surprisingly and in contrast to published reports, Experiment 1 indicated that nicotine attenuated startle amplitude and impaired pre-pulse inhibition in Long-Evans subjects. The purposes of Experiment 2, therefore, were to replicate and extend Experiment 1 results by examining ASR and PPI responses to nicotine administration in Sprague-Dawley vs. Long-Evans subjects within the same study. In order to investigate whether the two strains had differently-shaped or positioned dose-response curves, Experiment 2 also included an intermediate nicotine dosage. Further, because housing effects in Experiment 1 were complex and not easily interpreted, Experiment 2 included immobilization stress in order to compare ASR and PPI responses of the two strains to this stressor and to examine this specific stress-nicotine interaction in two strains.

Experiment 1

Overview

This experiment examined effects of nicotine administration and cessation and environmental conditions on body weight, the acoustic startle reflex (ASR), and pre-pulse inhibition (PPI) of the ASR. The subjects were 192 Long-Evans rats, 96 males and 96 females. The experiment proceeded in three phases: Baseline Phase (pre-drug, pre-environmental manipulation), Drug Administration Phase (during drug administration and environmental manipulation), and Drug Cessation Phase (after drug cessation with continued environmental manipulation). The experimental design was a 2 (male or female) X 2 (saline or 12 mg/kg/day nicotine) X 2 (individual or crowded housing) X 2 (Drug Administration Phase or Drug Cessation Phase) design with 12 subjects per cell (Table 1).

Hypotheses

There were five hypotheses. **Hypotheses 1**, **2**, and **3** were based on previous reports. **Hypotheses 4** and **5** were original.

Hypothesis 1: It was hypothesized that nicotine administration would decrease body weight and that these effects would be greater in females than in males.

Rationale: Previous studies (Grunberg, 1982; Grunberg, 1986; Grunberg et al., 1991) have established that nicotine decreases body weight and that these effects are greater in females than in males.

Hypothesis 2: It was hypothesized that nicotine administration would increase startle amplitude and increase amount of pre-pulse inhibition.

Rationale: Previous studies (Acri et al., 1991; Acri, 1992, 1994; Popke et al.,

1994; Acri et al., 1994; Acri et al., 1995) using Sprague-Dawley subjects reported that nicotine administration increased startle amplitude regardless of administration route. Curzon et al. (1994) reported that acute nicotine administration had no effect on male Long-Evans' startle responses. Time of testing as well as other procedural differences (e.g., nicotine form), however, limit the comparability of these studies. Therefore, it was hypothesized that chronic nicotine administration would enhance Long-Evans subjects' responses when measured during the dark cycle. Effects of chronically-administered nicotine in females are not known. Therefore, it was hypothesized that chronic administration would enhance startle responses of female subjects.

With regard to PPI, previous studies (Acri, 1992, 1994; Acri et al., 1992; Acri et al., 1994; Popke et al., 1994) using male and female Sprague-Dawleys reported that nicotine administration increased PPI whether administered acutely or chronically. In Long-Evans males Curzon et al. (1994) reported enhanced PPI as a result of acute nicotine administration. Responses of Long-Evans females have not been studied but were hypothesized to be similar to Sprague-Dawley female responses.

Hypothesis 3: It was hypothesized that nicotine cessation would have no effect on ASR amplitudes and would decrease PPI amounts.

Rationale: Acri et al. (1991) and Acri (1992) found that ASR amplitudes returned to baseline during nicotine cessation. In contrast, Helton et al. (1993) and Rasmussen et al. (1996) reported that, in Long-Evans subjects, startle amplitudes increased in nicotine cessation. Methodological differences among studies, however, limit their comparability. With regard to PPI, Acri (1992) reported that nicotine cessation decreased PPI.

Hypothesis 4: It was hypothesized that stress would increase startle and PPI in males and decrease these responses in females.

Rationale: Previous studies (Acri, 1992, 1994) in male Sprague-Dawley subjects reported that restraint stress increased startle amplitudes. Brown and Grunberg (1995) reported that, in Wistar subjects, males were stressed by crowded housing and females were stressed by individual housing. Crowded housing, therefore, was predicted to stress male subjects and to increase startle amplitudes. Acri (1992, 1994) reported that restraint stress increased PPI. In the present experiment crowded housing was conceptualized as a stress condition for males (Brown & Grunberg, 1995). Therefore crowded saline males were predicted to have greater PPI amounts than individually-housed saline males.

With regard to females, Brown and Grunberg (1995) reported that individually-housed females were stressed when compared to crowded females. Popke et al. (1994) reported that restraint stress reduced startle amplitude and PPI of Sprague-Dawley females. Therefore, it was predicted that individually housed saline females represented the stressed condition and would have reduced startle amplitudes and PPI when compared with crowded saline females.

Hypothesis 5: It was hypothesized that nicotine would interact with environmental stress such that stressed subjects receiving 12 mg/kg/day nicotine would exhibit startle and PPI responses indistinguishable from non-stressed saline controls.

Rationale: Acri (1992, 1994) found that male Sprague-Dawley rats administered 12 mg/kg/day nicotine and subjected to restraint stress had startle amplitudes similar to saline non-stressed subjects. If crowded males are conceptualized as stressed, then

nicotine males in the crowded housing condition should exhibit responses similar to 12 mg/kg/day nicotine subjects that experienced restraint stress. In addition, Acri (1992, 1994) found that animals administered 12 mg/kg/day nicotine and subjected to restraint stress had pre-pulse inhibition levels similar to saline-treated controls. Following from Brown and Grunberg (1995), the present experiment hypothesized that crowded housing would stress males and individual housing would be a non-stress condition. Therefore, following from Acri (1992), crowded males (stressed subjects) receiving 12 mg/kg/day nicotine were predicted to have PPI levels similar to individually-housed saline subjects.

With regard to females, the present experiment conceptualized the individually-housed condition as stressful based on Brown and Grunberg (1995). Therefore, individually-housed females receiving 12 mg/kg/day nicotine were predicted to have ASR amplitudes similar to crowded females (non-stress group) receiving saline. Further, based on Acri's (1992, 1994) findings that stressed male 12 mg/kg/day nicotine subjects had PPI levels similar to non-stressed subjects receiving saline, it was predicted that individually-housed females (stressed group) receiving 12 mg/kg/day would exhibit PPI responses similar to crowded females (non-stressed group) receiving saline. No data were available to suggest that Long-Evans subjects would respond differently to this environmental manipulation than albino strains.

Methods

Subjects and Baseline Phase Housing

Subjects were 96 male and 96 female Long-Evans rats (Charles River Laboratories, Wilmington, MA). During the Baseline Phase (pre-drug and pre-housing manipulation) all animals were individually housed in standard polypropylene shoebox cages (42 x 20.5 x 20 cm) on hardwood chip bedding (Pine-Dri). Throughout the study animals had continuous access to rodent chow (Harlan Teklad 4% Mouse/Rat Diet 7001) and water. Housing rooms were maintained at 23° C at 50% relative humidity on a 12-hour reverse light/dark cycle (lights on at 1900 hours). At the beginning of the experiment, subjects were approximately 51-55 days old. At the beginning of the experiment, males weighed approximately 234 g; females weighed approximately 194 g.

Equipment

Acoustic startle reflex amplitudes and pre-pulse inhibition were measured in a Coulbourn Instruments Acoustic Response Test System (Coulbourn Instruments, Allentown, PA). The Acoustic Response Test System consists of four weight-sensitive platforms inside a sound-attenuated chamber. Subjects' movements in response to stimuli are measured as a voltage change by a strain gauge inside each platform and are converted to grams of body weight change following analog to digital conversion. Responses are recorded by an interfaced computer as the maximum response occurring within 200 msec of the onset of the startle-eliciting stimulus. Each rat was individually placed in a 8 x 8 x 16 cm open air cage. The open air cages were small enough to restrict extensive locomotion but large enough to allow the subject to turn around and make other small

movements. Each open air cage was then placed on one of the four platforms. The platforms were arranged radially around central speakers in the floor and ceiling of the chamber. A ventilating fan provided an ambient noise level of 56dB. Following placement of four animals in the chamber, a 3-minute adaptation period occurred in which no startle stimuli were presented. Although it has been reported that some rats emit ultrasonic vocalizations during startle testing (Miczek, Vivian, Tornatsky, Farrell, & Sapperstein, 1992), there is no evidence indicating that vocalizations alter startle responses. However, to ensure minimal effects of vocalizations should they occur, subjects were balanced across treatment groups within each testing session.

Startle stimuli consisted of 112 or 122dB noise bursts of 20 msec duration sometimes preceded 100 msec by 68dB white noise bursts (pre-pulses). Decibel levels were verified by a Larson-Davis Sound Pressure Machine Model 2800 (unweighted scale; re: 0.0002 dynes/cm^2). Each stimulus had a 2 msec rise and decay time such that onset and offset were abrupt, a primary criterion for startle. There were six types of stimulus trials, and each trial type was presented eight times, for a total of 48 trials. Trial types were presented in random order to avoid order effects and habituation. Inter-trial intervals ranged randomly from 10 - 30 sec. Trial types included: (1) 112dB stimulus, (2) 112dB stimulus preceded by a 68dB pre-pulse, (3) 122dB stimulus, (4) 122dB stimulus preceded by a 68dB pre-pulse, (5) 68dB pre-pulse only, and (6) no stimulus. The testing period lasted approximately 15 min. Open-air cages were washed with warm water and dried after each use. Males and females were tested in separate test chambers. Treatment groups were balanced within each chamber for each measurement session.

Trials during which no stimuli were presented were used to control for normal subject movements on the platform. This information is necessary in order to accurately calculate platform displacement that occurs in response to specific noise stimuli. To derive these values, platform displacement on the no-stimulus trials (i.e., the body weight of each subject) was subtracted from platform displacement in response to the various noise stimuli, leaving only the amount of platform displacement related to the stimulus.

Drug Administration and Surgical Procedure

Nicotine (12 mg/kg/day) or physiologic saline was administered via Alzet osmotic mini-pumps (Model 2002, Alza Corp., Palo Alto, CA). Physiological saline also was used as vehicle for the nicotine solution. Nicotine solution was made from nicotine dihydrochloride. The concentration of 12 mg/kg/day is expressed as nicotine base. Minipumps administered nicotine or saline solution at a rate of approximately 0.47 μl/hr. Dosages were calculated based on body weight such that nicotine-treated animals received 12 mg/kg/day. This method of drug administration was chosen because it avoids the repeated stress of daily injections, and has produced results in rats that have been replicated in studies of human smokers (Grunberg, 1982; Winders & Grunberg, 1989). This drug dose has produced behavioral effects in rats that approximate those in human smokers (Grunberg, 1986; Grunberg, Bowen, & Morse, 1984). In addition, this dose and route of administration has produced reliable changes in ASR and PPI (Acri, 1992; Acri et al., 1991).

Subjects were anesthetized using methoxyflurane (Metofane) in a bell jar inside a vented hood. Subjects were removed from the bell jar when tail pinch produced no reflex

movement. Then, a 3 x 5 cm area between the withers was shaved and cleaned with Betadine. A 2 cm transverse incision within the shaved region was made with blunt-nosed surgical scissors, a subcutaneous pocket was created by spreading the subcutaneous tissues with the scissor tips, and the minipump was inserted with the flow modulator toward the subject's head. The incision was closed with 9 mm stainless steel wound clips. The entire surgical procedure including anesthesia took approximately 4 minutes.

Cessation Phase subjects also underwent explant of minipumps. Anesthesia was administered as described above. A 2.5 x 4 cm area surrounding the implanted minipump was shaved and cleaned with Betadine. A 1.5 cm incision was made at the base of the implanted minipump and the minipump was removed. The incision was closed with 9 mm stainless steel wound clips. The entire surgical procedure including anesthesia took approximately 3 minutes.

Environmental Manipulation

During the Baseline Phase all subjects were individually housed in standard shoebox cages. Individual housing was maintained during the baseline phase to ensure comparability with other relevant studies (Acri et al., 1991; Acri, 1992, 1994; Acri et al., 1994; Popke et al., 1994; Acri et al., 1995; Helton et al., 1993; Curzon et al., 1994; Rasmussen et al., 1996), all of which housed subjects individually. At the beginning of the Drug Administration Phase, subjects were assigned to an individual or crowded housing condition in a manner that insured comparable body weights between conditions. Individual or crowded housing was established based on the procedures of Brown and Grunberg (1995) in order to produce environmental conditions that have been reported to

alter behavioral and biochemical responses of rats (Brown & Grunberg, 1995, 1996). Specifically, one day after surgery animals in the individually-housed condition were transferred to clean standard shoebox cages. Crowded subjects were placed in same-sex groups of six. For crowded subjects floor space per animal was adjusted based on mean body weights to provide approximately 55% of U.S. Department of Health and Human Services (USDHHS) recommended floor space per animal.

USDHHS floor space recommendations are based on body weight ranges (e.g., 100-200 g, 200-300 g, 300-400 g), with larger animals recommended to have more space. Therefore, because of different mean body weights, males and females required cages with different amounts of floor space. Crowded males ($\bar{x}$ = 292.8 g) were placed in standard shoebox cages (six subjects per cage). This cage size provided approximately 143.5 cm^2 of floor space per male subject (55% of USDHHS recommended floor space for weight range 300-400 g). Crowded females ($\bar{x}$ = 210.4 g) also were placed in standard shoebox cages (six subjects per cage), and the amount of floor space was adjusted using a polypropylene divider bolted to the cage top. The divider was placed so that each female subject had approximately 102.9 cm^2 of floor space (55% of USDHHS recommended floor space for weight range 200-300 g). Individually-housed animals had cages changed twice a week. Crowded subjects' cages were changed every other day and were checked twice daily to insure that subjects had adequate food and water.

Procedure

Table 2 presents the timeline for Experiment 1. The procedure included three phases: a pre-drug, pre-housing manipulation (Baseline Phase), a during drug

administration and housing manipulation phase (During Drug Phase), and a drug cessation phase in which drug administration ceased but the housing manipulation continued (Cessation Phase). Subjects' body weights were measured every third day throughout all three phases. ASR-PPI were measured for all subjects (N=192) during the Baseline Phase and on Day 6 of the During Drug Phase. Half of the subjects had ASR-PPI measured again on Day 11 of the During Drug Phase (During Phase subjects; n = 96), whereas the other half of the subjects had ASR-PPI measured on Day 3 of the Cessation Phase (Cessation Phase subjects; n = 96). The During Phase subjects were sacrificed at the end of the During Drug Phase; the Cessation Phase subjects were sacrificed at the end of the Cessation Phase. The different ASR-PPI timing for each group was necessary so that groups had the same total number of ASR-PPI exposures.

Baseline Phase. Subjects were gentled once each day for three days. After gentling, subjects underwent an acclimation exposure to the ASR-PPI procedure and baseline ASR-PPI was measured. For the ASR-PPI acclimation, subjects were placed in the open-air cages inside the test chamber and were exposed to the noise stimuli. Three days after the acclimation exposure ASR-PPI baseline responses were measured. The Baseline Phase lasted approximately two weeks.

Drug Administration Phase. After the completion of baseline measures, subjects were assigned within sex to drug (0 mg/kg/day or 12 mg/kg/day nicotine), housing (individual or crowded), and phase (during nicotine administration or nicotine withdrawal) groups in a manner that assured comparable, initial body weights. This assignment resulted in 16 balanced groups of 12 subjects each (8 groups of males; 8 groups of

females). Minipumps containing the appropriate solutions were implanted as described in Drug Administration and Surgical Procedure on During Drug Phase day 1. Twenty-four hours after surgery, subjects were placed in their assigned housing condition (individual or crowded). Crowded animals were observed continuously for the first hour of crowding, and were checked hourly for the following 3 hours.

ASR-PPI was measured on During Drug Phase day 6 (after 5 days of saline or nicotine administration and 4 days of individual or crowded housing) for all subjects (N = 192). ASR-PPI was measured again on During Drug Phase day 12 (after 11 days of saline or nicotine administration and 10 days of individual or crowded housing) for During Phase subjects (n = 96). During Phase subjects were sacrificed without anesthesia on During Drug Phase day 13 and blood and brains were collected and stored for purposes of other experiments.

Cessation Phase. Cessation Phase subjects (n = 96) had minipumps explanted on During Drug Phase day 15 as described in Drug Administration and Surgical Procedure. ASR-PPI was measured for these subjects on the third day of nicotine or saline cessation (after 16 days of individual or crowded housing). Cessation Phase subjects were sacrificed without anesthesia on Drug Cessation Phase day 5. Blood and brains were collected and stored for other experiments.

Results

Data Analytic Strategy

Body Weight. Body weight data were analyzed by repeated-measures analysis of covariance (ANCOVA) with average baseline body weights as covariates. Separate analyses were conducted for During Phase males and females and Cessation Phase males and females, with time as the within-subject factor, and drug and housing condition as between-subjects factors for all analyses. Males and females were analyzed separately because significant body weight differences existed between the sexes at all time points and empirical literature indicates that effects of nicotine on body weight are greater in females than in males. Phases were analyzed separately in order to include Cessation Phase body weight data for Cessation Phase subjects. For During Phase subjects (males and females), the analysis included five time points: Baseline Day 4, During Drug Phase Days 1, 6, 11, and 13. For Cessation Phase subjects the analysis included Baseline Day 4, During Drug Phase Days 1, 6, 13, and 15, and Cessation Day 3 for a total of six time points. Subsequent ANCOVAs with baseline body weights as covariates and factors of drug and housing condition were conducted at each time point to determine which groups differed significantly. All tests were two-tailed with alpha ≤ 0.05.

ASR-PPI. Startle amplitudes to each stimulus (112 and 122dB) were calculated by subtracting the amount of platform displacement in g on the no-stimulus trials (i.e., the body weight of each subject) from the amount of platform displacement in response to each stimulus for each subject at each time point. The remainders were analyzed as startle amplitude. Amount of pre-pulse inhibition was calculated by subtracting the amount of

platform displacement in g on no-stimulus trials from each stimulus (112 and 122dB) when presented with pre-pulse. This amount, representing startle amplitude to each stimulus with pre-pulse, was then subtracted from the startle amplitude to each stimulus (112 and 122dB) without pre-pulse. This calculation was done for each subject at each time point. The remainders were analyzed as amount of pre-pulse inhibition. Percent pre-pulse inhibition was calculated by expressing amount of pre-pulse to each stimulus (112 and 122dB) with pre-pulse as a percentage of startle amplitude. Specifically, amount of pre-pulse for each stimulus was multiplied times 100 and divided by startle amplitude to that stimulus without pre-pulse. This calculation was done for each subject at each time point. The products were analyzed as percent pre-pulse inhibition. These calculations were based on the procedures of Acri (1992, 1994), Acri et al. (1994, 1995), and Swerdlow et al. (1986, 1990, 1992, 1994).

An initial multivariate analysis of variance (MANOVA) was performed that included startle amplitudes (112dB and 122dB) without pre-pulse and startle amplitudes (112dB and 122dB) with pre-pulse from the Baseline Phase and During Drug Phase day 6 to determine whether stimulus intensity and stimulus type (i.e., pre-pulse or no pre-pulse) significantly affected responses. This analysis indicated that responses were significantly different to each stimulus intensity and stimulus type. Therefore, the data for each stimulus with and without pre-pulse were analyzed separately.

In general, each stimulus with and without pre-pulse was analyzed in a series of analyses of covariance (ANCOVAs). The first ANCOVA was done as an overall model with all factors included. Subsequent ANCOVAs were done on males and females

separately, and on individually-housed and crowded animals separately. Additional ANCOVAs were done within sex and housing condition. In the **RESULTS** text, **findings reported are significant at the $p \leq 0.05$ level unless otherwise noted**. Findings for specific subgroups are explicitly designated for clarity (e.g., "for individually-housed subjects" or "for males").

More specifically, analyses were conducted as follows. Three-way analyses of covariance (ANCOVAs) were performed on ASR amplitudes to each stimulus (112 and 122dB) with drug, housing, and sex as separate factors on Day 6 of drug administration for all subjects (N = 192) using baseline responses as covariates. Similar analyses were performed on amount of pre-pulse inhibition and percent pre-pulse inhibition in response to each stimulus (112 and 122dB) with pre-pulse on Day 6 using baseline responses as covariates. Baseline covariates were used because preliminary analyses indicated significant baseline differences among some but not all subgroups. Baseline differences on these measures are consistent with past work finding significant individual differences in ASR and PPI responses before experimental manipulations (Acri, 1992).

In addition, ANCOVAs were conducted on males and females separately on Day 6 measures because of *a priori* hypotheses based on empirical reports that females are more sensitive to the effects of nicotine than males and that males and females respond differentially to housing conditions. Also, separate ANCOVAs were conducted on Day 6 responses of individually-housed and crowded subjects (collapsing across sex) because of *a priori* hypotheses that housing condition would affect responses. Finally, ANCOVAs were conducted on Day 6 responses of male and female individually-housed and crowded

subjects separately because of *a priori* hypotheses that sex and housing condition would interact. Similar analyses were conducted for Day 11 ASR and PPI responses for the During Phase subjects (n = 96), and on Day 3 of withdrawal for the Withdrawal Phase subjects (n = 96). One-way ANOVAs were used where necessary to do planned comparisons between groups in order to confirm specific hypotheses.

Results: Body Weight

Figures 1 and 2 present body weights in grams of male and female During Phase (i.e., those subjects that were terminated at the end of the drug administration phase) and Cessation Phase (i.e., those subjects that were terminated in the nicotine cessation phase) subjects at multiple time points. Nicotine-treated subjects weighed less than saline-treated subjects regardless of phase or sex {During Phase males [F (1, 43) = 29.167] and females [F (1, 43) = 26.180], Cessation Phase males [F (1, 42) = 15.321] and females [F (1, 43) = 58.011]}. In addition, crowded housing reduced body weights of Cessation males [F (1, 42) = 5.099] and tended to reduce body weights of Cessation females [F (1, 43) = 3.228, p = 0.079].

Drug and housing condition also altered body weight *over time*. Specifically, nicotine-treated subjects' body weights increased less over time than did saline-treated subjects' body weights regardless of sex or phase {During males [F (4, 172) = 37.274] and females [F (4, 172) = 9.912], Cessation males [F (5, 210) = 17.488] and females [F (5, 215) = 24.782]}. Crowding also decreased body weight gains over time for all subjects {During males [F (4, 172) = 3.287] and females [F (4, 172) = 2.989], Cessation males [F (5, 210) = 5.499] and females [F (5, 215) = 2.220]}. In addition, nicotine reduced body

weight for During Phase females more over time in the crowded condition than in the individually-housed condition [F (4, 172) = 3.555].

Drug and housing also altered body weights on specific measurement days. Specifically, nicotine administration reduced body weights of all subjects on During Drug Day 6 {During males [F (1, 43) = 28.071] and females [F (1, 43) = 24.094], Cessation males [F (1, 42) = 16.967] and females [F (1, 43) = 61.881]} and Day 13 {During males [F (1, 43) = 49.941] and females [F (1, 43) = 19.263], Cessation males [F (1, 42) = 22.023] and females [F (1, 43) = 52.325]}. Nicotine administration also reduced: Day 11 body weights of During males [F (1, 43) = 37.294] and females [F (1, 43) = 30.554], Day 15 body weights of Cessation subjects {males [F (1, 42) = 32.328] and females [F (1, 43) = 82.147]}, and Cessation Day 3 body weights {males [F (1, 42) = 7.299] and females [F (1, 43) = 27.193]}.

Crowded housing reduced body weights of During females on Day 11 [F (1, 43) = 6.747] and also interacted with Drug such that crowding reduced body weight more in nicotine-treated subjects than in saline-treated subjects [F (1, 43) = 4.172]. Crowding reduced body weights of Cessation Phase males on Day 6 [F (1, 4) = 10.088] and Day 15 [F (1, 42) = 6.102], and reduced Cessation Day 3 body weights of males [F (1, 42) = 4.889] as well as females [F (1, 43) = 5.368].

Results: ASR-PPI in *During Drug Phase*

Startle Amplitude to 112dB. Figure 3 presents startle amplitude in g to the 112dB stimulus without pre-pulse on Experimental Days 6 and 11. Nicotine-treated animals startled **less** than did saline-treated animals [F (1, 176) = 4.262] on Day 6. This

pattern was evident for females [F (1, 87) = 7.081], for individually-housed subjects regardless of sex [F (1, 89) = 5.118], and for female individually-housed subjects [F (1, 43) = 9.401]. By Day 11 these effects were still evident in females but were reversed in males (F (1, 85) = 3.688, p = 0.058], with nicotine tending to decrease startle in females, but to increase startle amplitude in males. This interaction also was clear in crowded subjects' responses [F (1, 42) = 4.165]. In addition, on Day 11 crowded housing increased startle amplitude (F (1, 85) = 6.599], especially in female subjects [F (1, 42) = 4.552] as well as in saline-treated females [F (1, 21) = 3.984, p = 0.059].

Startle Amplitude to 122dB. Figure 4 presents startle amplitude in g to the 122dB stimulus without pre-pulse on Experimental Days 6 and 11. Males startled more than did females on Day 6 [F (1, 176) = 7.901]. This pattern was clear for crowded subjects [F (1, 86) = 4.252] and evident to a lesser extent in individually-housed subjects [F (1, 89) = 3.117, p = 0.081]. On Day 11 crowded housing tended to increase startle for all subjects [F (1, 85) = 3.850, p = 0.053]. Nicotine's effects depended on housing condition with nicotine tending to decrease startle in individually-housed subjects but increase startle in crowded subjects [F (1, 85) = 3.664, p = 0.059]. This pattern was clear in male responses [F (1, 42) = 5.173] and nicotine's startle-reducing effects also were evident for individually-housed males [F (1, 21) = 3.956, p = 0.060].

Amount of Pre-Pulse Inhibition to 112dB w/ Pre-Pulse. Figure 5 presents amount of pre-pulse inhibition in g to the 112dB stimulus with pre-pulse on Experimental Days 6 and 11. On Day 6 nicotine **decreased** amount of inhibition in individually-housed subjects and slightly **increased** inhibition in crowded subjects [F (1, 176) = 4.355],

especially for male subjects [F (1, 88) = 3.156, p = 0.079]. In contrast to male responses, nicotine **decreased** female PPI regardless of housing condition [F (1, 87) = 9.618]. Nicotine-induced PPI reductions also were evident in individually-housed subjects' responses [F (1, 89) = 8.958], especially in responses of individually-housed females [F (1, 43) = 8.321]. PPI reductions as a result of nicotine administration also were evident on Day 11 [F (1, 85) = 3.950, p = 0.050], especially for females [F (1, 42) = 4.596], and more specifically, for individually-housed females [F (1, 20) = 4.372]. In addition for females nicotine administration and individual housing decreased PPI when compared to responses of saline-treated crowded females [T (1, 44) = 2.542].

Amount Pre-Pulse Inhibition to 122dB w/ Pre-Pulse. Figure 6 presents amount of pre-pulse inhibition (PPI) in g to the 122dB stimulus with pre-pulse on Experimental Days 6 and 11. On Day 6 males exhibited greater amounts of pre-pulse inhibition than did females [F (1, 176) = 12.201] whether subjects were individually-housed [F (1, 89) = 6.603] or crowded [F (1, 86) = 5.777]. On Day 11 nicotine administration tended to decrease individually-housed subjects' PPI amounts but increase crowded subjects' PPI [F (1, 85) = 3.898, p = 0.052]. PPI reductions as a result of nicotine administration also were evident for individually-housed subjects [F (1, 42) = 5.421].

Percent Pre-Pulse Inhibition to 112dB w/ Pre-Pulse. Figure 7 presents percent pre-pulse inhibition (PPP) to the 112dB stimulus with pre-pulse on Experimental Days 6 and 11. Nicotine administration **reduced** percent pre-pulse inhibition on Day 6 [F (1, 176) = 6.044] and also interacted with housing condition such that nicotine **decreased** PPP in individually-housed subjects but not in crowded subjects [F (1, 176) = 5.437].

Nicotine-induced PPP reduction was evident in female responses regardless of housing condition [F (1, 87) = 7.750], in individually-housed subjects regardless of sex [F (1, 89) = 8.904], in individually-housed males [F (1, 45) = 4.550] and females [F (1, 43) = 4.175], and in crowded females [F (1, 43) = 3.745, p = 0.060]. In contrast, the interaction was evident in male responses [F (1, 88) = 5.384] with nicotine increasing PPP in crowded males but decreasing PPP in individually-housed males. For crowded subjects nicotine administration increased PPP in males but decreased PPP in females [F (1, 86) = 4.081]. Similar patterns were evident in Day 11 responses. Nicotine-treated subjects tended to exhibit **less** PPP than saline-treated subjects [F (1, 85) = 3.845, p = 0.053], especially in crowded housing conditions [F (1, 42) = 3.706, p = 0.061].

Percent Pre-Pulse Inhibition to 122dB. Figure 8 presents percent pre-pulse inhibition to the 122dB stimulus with pre-pulse on Experimental Days 6 and 11. On Day 6 males exhibited greater PPP than females [F (1, 176) = 5.380], especially in the individual housing condition [F (1, 89) = 4.674], and individually-housed subjects exhibited greater PPP than crowded subjects [F (1, 176) = 4.515]. Saline-treated individually-housed males also had greater PPP levels than nicotine-treated crowded males [F (1, 90) = 2.10]. There were no significant findings on this measure on Day 11.

Results: ASR-PPI in *Cessation Phase*

Startle Amplitude to 112dB. Figure 9 presents startle amplitude in g to the 112dB stimulus without pre-pulse on Cessation day 3. Analyses revealed no significant findings.

Startle Amplitude to 122dB. Figure 10 presents startle amplitude in g to the

122dB stimulus without pre-pulse on Cessation day 3. Males startled more than did females [F (1, 87) = 5.264].

Amount Pre-Pulse Inhibition to 112dB w/ Pre-Pulse. Figure 11 presents amount of pre-pulse inhibition in g to the 112dB stimulus with pre-pulse on Cessation day 3. Analyses revealed no significant findings.

Amount Pre-Pulse Inhibition to 122dB w/ Pre-Pulse. Figure 12 presents amount of pre-pulse inhibition in g to the 122dB stimulus with pre-pulse on Cessation Day 3. Males exhibited greater PPI than females [F (1, 87) = 7.642], especially in individually-housed subjects [F (1, 43) = 5.117].

Percent Pre-Pulse Inhibition to 112dB w/ Pre-Pulse. Figure 13 presents percent pre-pulse inhibition to the 112dB stimulus with pre-pulse on Cessation day 3. Individually-housed males exhibited more PPP than crowded males, but crowded females exhibited greater PPP than individually-housed females [F (1, 87) = 6.101]. The effects of crowding to decrease PPP in males [F(1, 21) = 4.169, p = 0.054] but increase PPP in females [F (1, 43) = 4.224] also were clear when the sexes were analyzed separately. In addition, individually-housed males exhibited greater PPP than individually-housed females [F (1, 43) = 5.823]. For crowded subjects regardless of sex, nicotine-cessation tended to produce greater PPP than saline-cessation [F (1, 43) = 3.850, p = 0.056].

Percent Pre-Pulse Inhibition to 122dB w/ Pre-Pulse. Figure 14 presents percent pre-pulse inhibition to the 122dB stimulus with pre-pulse on Cessation day 3. Nicotine-cessation subjects tended to exhibit less PPP than saline-cessation subjects [F (1, 87) = 3.643, p = 0.060].

Confirmation of Hypotheses

Hypothesis 1 that nicotine administration would decrease body weight was **confirmed**. The hypothesis that these effects would be greater in females than in males was **not confirmed**. Nicotine significantly reduced body weight in males and females regardless of housing condition, however, the magnitude of these effects was similar in males and females.

Hypothesis 2 that nicotine administration would increase startle amplitude and amount of pre-pulse inhibition was mostly **disconfirmed**. Nicotine administration **decreased** startle amplitude and **decreased** amount and percent of pre-pulse inhibition in female subjects regardless of housing condition on Days 6 and 11. For male subjects that were individually-housed, nicotine administration had no effect on startle amplitude on Day 6, and decreased startle amplitude on Day 11. Nicotine's effects on pre-pulse inhibition showed the opposite pattern for these subjects, decreasing amount and percent PPI on Day 6, and having no effect on PPI on Day 11. For crowded male subjects, however, nicotine administration had no effect on Day 6 startle amplitude but increased Day 11 amplitude. Nicotine administration also increased PPI for these subjects on Day 6 but decreased PPI on Day 11.

Hypothesis 3 that nicotine cessation would have no effect on ASR amplitudes and would decrease PPI amounts was **partially confirmed**. Specifically, nicotine cessation had no effect on startle amplitude or on amounts of pre-pulse inhibition. Effects of nicotine cessation on percent pre-pulse inhibition (PPP) depended on housing condition and stimulus intensity. Specifically, nicotine cessation increased PPP in response to the

112dB stimulus for crowded subjects. This increase represents a reversal and overshoot of nicotine administration effects. In response to the 122dB stimulus, however, nicotine cessation resulted in a trend toward continued, decreased PPP for all subjects.

Hypothesis 4 that stress -- conceptualized as crowded housing for males and individual housing for females -- would increase startle amplitude and pre-pulse inhibition in males and decrease these responses in females was **not confirmed**. There were no differences in startle amplitude or pre-pulse amounts of saline-treated males and females that were the result of housing conditions with the exception of two trends. On Day 11 there was a trend ($p = 0.059$) for crowded saline-treated females to startle more to the 112dB stimulus than individually-housed saline-treated females. The direction of this trend was consistent with the hypothesized effect of stress for females (i.e., that individually-housed females would have reduced startle amplitudes when compared with crowded females) but was present at only one time point. The second trend ($p = 0.054$) occurred in saline-treated males in cessation. Specifically, individually-housed saline-treated males tended to exhibit greater percent pre-pulse inhibition than crowded saline-treated males to the 112dB stimulus. This trend was opposite of the hypothesized effect of stress for males.

Hypothesis 5 that stress and nicotine would interact such that stressed subjects receiving 12 mg/kg/day nicotine would exhibit startle and PPI responses indistinguishable from non-stressed controls was **partially confirmed**. Specifically, there were no differences between responses of saline-treated individually-housed (non-stressed) males and nicotine-treated crowded (stressed) males with the exception of PPP responses on

Day 6 to the 122dB stimulus. On this day saline-treated individually-housed males had significantly greater PPP than did nicotine-treated crowded males. Further, there were no differences between responses of saline-treated crowded (non-stressed) females and nicotine-treated individually-housed (stressed) females with the exception of pre-pulse amounts on Day 11 to the 112dB stimulus. On this day saline-treated crowded females had significantly greater pre-pulse amounts than did nicotine-treated individually-housed females. Although groups conceptualized as "no stress" (saline-treated individually-housed males; saline-treated crowded females) generally exhibited responses indistinguishable from groups conceptualized as "stress + nicotine" (nicotine-treated crowded males; nicotine-treated individually-housed females), other groups also exhibited responses indistinguishable from these two groups. Therefore, the form of the hypothesis is supported, but its substance is mostly not supported.

Discussion of Experiment 1

In contrast to reported findings in Sprague-Dawley subjects (Acri et al., 1991; Acri, 1992, 1994; Acri et al., 1994; Popke et al., 1994; Acri et al., 1995), nicotine decreased startle amplitude and impaired pre-pulse inhibition in Long-Evans subjects in the present experiment. These drug effects occurred for females regardless of housing condition. For males nicotine's effects on responses depended on housing condition, with nicotine reducing responses for individually-housed males and increasing responses for crowded males. These effects were clearest in response to the 112dB stimulus.

Nicotine administration and crowding separately decreased body weights of Long-Evans males and females. The effects of nicotine administration are consistent with previous reports in Sprague-Dawley subjects but contrast with previous reports in Long-Evans subjects in which a different chemical form of nicotine was used and subjects did not appear to receive nicotine based on a failure to lose weight (Helton et al., 1993). Further, the housing effects suggest that males may be more sensitive to the environmental manipulation of crowding than are females. This sensitivity to environmental conditions is consistent with males' startle and PPI data where drug effects depended on subjects' housing condition.

Experiment 2 was designed to replicate and extend Experiment 1 by examining effects of nicotine on ASR and PPI responses in Sprague-Dawley and Long-Evans male and female subjects within the same study. To clarify the results of Experiment 1 with regard to nicotine's effects and stress' effects, several changes were made to Experiment 2: **1)** two dosages of nicotine were included in Experiment 2 (6 and 12 mg/kg/day); **2)** the

cessation phase was not included; **3)** immobilization was used as a stressor; **4)** a 98dB stimulus was used in addition to the 112dB and 122dB stimuli; and, **5)** an additional ASR-PPI measurement was done 24 hours after minipump implant. The rationales for these changes are described below.

1) Like many drugs nicotine exerts biochemical, physiological, and behavioral effects in an inverted-U-shaped dose-response curve (USDHHS, 1988). That is, these responses increase at low doses of nicotine but decrease at higher doses. Opposite behavioral effects as a result of the same drug dosage, therefore, may indicate that one strain is more sensitive to nicotine than the other strain. That is, one strain's inverted U-shaped dose-response curve may be shifted to the left of the other strain's curve. Decreased responses as a result of treatment with 12 mg/kg/day nicotine suggested that Long-Evans subjects might be more sensitive to nicotine's effects than Sprague-Dawley subjects. This decrease might occur because 12 mg/kg/day represented a dosage on the descending limb of the Long-Evans dose-response curve but a point on the ascending limb of the Sprague-Dawley curve. In Experiment 1, however, the use of one dosage of nicotine (12 mg/kg/day) did not allow full extrapolation of the Long-Evans dose-response curve. Therefore, both 6 mg/kg/day and 12 mg/kg/day dosages were used in Experiment 2 in addition to the saline control group.

2) Because nicotine cessation effects on ASR and PPI were minimal in Experiment 1, the cessation phase was dropped from Experiment 2.

3) Further, because ASR and PPI responses to the housing manipulation were not consistent within each sex and across measures as predicted, it was difficult to determine

which behaviors might reflect responses altered as the result of stress. Therefore, Experiment 2 used because immobilization as a stressor because this procedure has produced similar stress-related biochemical changes in males and females and has consistently produced altered ASR and PPI in Sprague-Dawley subjects.

4) The 98dB stimulus was added because strain differences in baseline startle amplitudes and PPI have been reported (Acri et al., 1995).

5) Subjects' responses were measured 24 hours after implant as well as on Day 6 and Day 12 in order to capture short-term effects of nicotine administration and of stress.

Experiment 2

Overview

This experiment examined effects of two dosages of nicotine (6 and 12 mg/kg/day) as well as saline and restraint stress on body weight, the acoustic startle reflex (ASR), and pre-pulse inhibition (PPI) of the ASR in male and female rats of two strains. Subjects were 120 Sprague-Dawley rats (60 male, 60 female) and 120 Long-Evans rats (60 male, 60 female). The experiment included two phases: Baseline Phase (pre-drug, pre-stress manipulation) and During Drug Phase (during drug and stress manipulation). The experiment was a 2 (Sprague-Dawley or Long-Evans) X 2 (male or female) X 2 (no stress or stress) X 3 (0, 6 or 12 mg/kg/day nicotine) design with 10 subjects per cell (Table 3).

Hypotheses

There were five hypotheses. **Hypotheses 1, 2**, and **5** were based on previous reports. **Hypothesis 3** was based on Experiment 1. **Hypothesis 4** was original.

Hypothesis 1: It was hypothesized that nicotine administration would decrease body weight in a dose-response fashion for all subjects, with 12 mg/kg/day nicotine decreasing body weight more than 6 mg/kg/day nicotine. It also was hypothesized that these effects would be greater in Sprague-Dawley females than in Sprague-Dawley males and of similar magnitude in Long-Evans males and females.

Rationale: Previous studies (Grunberg, 1982; Winders & Grunberg, 1989) have established that nicotine decreases Sprague-Dawley body weights in a dose-response manner and that these effects are greater in females. Experiment 1 indicated that nicotine reduced Long-Evans body weights without significant sex differences.

Hypothesis 2: It was hypothesized that Sprague-Dawley subjects would exhibit greater startle amplitudes and PPI amounts than would Long-Evans subjects.

Rationale: Acri et al. (1995) found that Sprague-Dawley male subjects exhibited greater startle amplitudes and PPI amounts than did Long-Evans male subjects.

Hypothesis 3: It was hypothesized that nicotine administration would increase startle amplitude and amount of pre-pulse inhibition in Sprague-Dawley subjects but decrease startle amplitude and amount of pre-pulse inhibition in Long-Evans subjects.

Rationale: Previous studies (Acri, 1992, 1994; Acri et al., 1991) found that 12 mg/kg/day nicotine via osmotic minipump increased startle amplitude and PPI in Sprague-Dawley male subjects. Nicotine administration via acute injection has been reported to enhance male and female Sprague-Dawley startle and PPI (Popke et al., 1994). With regard to Long-Evans subjects, Experiment 1 indicated that 12 mg/kg/day nicotine decreased startle amplitude and PPI in males and females of this strain.

Hypothesis 4: It was hypothesized that restraint stress would increase startle and PPI in Sprague-Dawley males and Long-Evans females, and decrease these responses in Sprague-Dawley females and Long-Evans males.

Rationale: Acri (1992, 1994) found that restraint stress increased startle amplitude and PPI in Sprague-Dawley males. With regard to Long-Evans subjects, no data are available that indicate effects of restraint stress on ASR and PPI. Acri (1992, 1994) reported, however, that stress and nicotine each produced similar behavioral responses in Sprague-Dawley male subjects (i.e., increased startle amplitudes and pre-pulse inhibition). Because effects of stress and effects of nicotine in Sprague-Dawley males resulted in

parallel behavioral responses, it was hypothesized that this relationship also would hold for Long-Evans male responses. Experiment 1 found that nicotine administration in Long-Evans subjects decreased startle and decreased pre-pulse inhibition. Therefore, it was predicted that Long-Evans males would respond to restraint stress in a manner similar to their responses to nicotine, and that stress would decrease ASR amplitude and PPI in saline-treated subjects.

With regard to female subjects, Popke et al. (1994) found that restraint decreased ASR and PPI responses of Sprague-Dawley females. Acri et al. (1994) and Popke et al. (1994) also found that nicotine administration enhanced ASR and PPI of Sprague-Dawley females. Therefore, in contrast to Sprague-Dawley males, for Sprague-Dawley females the behavioral effects of nicotine diverged from the behavioral effects of stress. It was hypothesized, therefore, that a similar relationship between nicotine administration and stress would hold for Long-Evans females. Specifically, because Experiment 1 indicated that nicotine administration decreased Long-Evans female ASR and PPI responses, it was hypothesized that restraint stress would increase these responses.

Hypothesis 5: It was hypothesized that restraint stress would combine with nicotine administration such that stressed nicotine-treated subjects would exhibit ASR and PPI responses similar to non-stressed saline-treated subjects.

Rationale: Acri (1992, 1994) found that male Sprague-Dawley rats administered 12 mg/kg/day nicotine and subjected to restraint stress had startle amplitudes and PPI similar to saline non-stressed subjects.

Methods

Subjects and Housing

Subjects included 120 Sprague-Dawley (60 male, 60 female) rats and 120 Long-Evans (60 male, 60 female) rats (Charles River Laboratories, Wilmington, MA). All animals were individually housed throughout the experiment in standard polypropylene shoebox cages (42 x 20.5 x 20 cm) on hardwood chip bedding (Pine-Dri). Throughout the study subjects had continuous access to rodent chow (Harlan Teklad 4% Mouse/Rat Diet 7001) and water. Housing rooms were maintained at 23° C at 50% relative humidity on a 12-hour reversed light/dark cycle (lights on at 1900). At the beginning of the experiment subjects were approximately 49 days old. At the beginning of the experiment males weighed approximately 228 g; females weighed approximately 172 g.

Equipment

The acoustic startle reflex and pre-pulse inhibition were measured using the equipment and procedures described in Experiment 1 (see Experiment 1, Equipment). Identical procedures were followed with the exception of the startle stimuli used. In addition to the 112dB and 122dB stimuli, a 98dB stimulus was added. The third stimulus was added because strain differences in startle amplitudes to these three stimuli have been reported (Acri, Brown, Saah, & Grunberg, 1995).

Startle stimuli of 98, 112, or 122dB noise bursts were sometimes preceded 100 msec by 68dB white noise bursts. There were eight types of stimulus trials, and each trial type was presented eight times, for a total of 64 trials. Trial types were presented in random order to avoid order effects and habituation. Trial types included: (1) 98dB

stimulus, (2) 98dB stimulus preceded by a 68dB pre-pulse, (3) 112dB stimulus, (4) 112dB stimulus preceded by a 68dB pre-pulse, (5) 122dB stimulus, (6) 122dB stimulus preceded by a 68dB pre-pulse, (7) 68dB pre-pulse only, and (8) no stimulus. The testing period lasted approximately 22 min. Open-air cages were washed with warm water and dried after each subject. Males and females were tested in separate test chambers. Same-sex treatment groups were balanced within each chamber for each measurement.

Drug Administration and Surgical Procedure

Nicotine (6 mg/kg/day or 12 mg/kg/day) or physiologic saline was administered using Alzet osmotic mini-pumps (Model 2002, Alza Corp., Palo Alto, CA). Physiological saline also was used as vehicle for the nicotine solution. Nicotine solution was made from nicotine dihydrochloride. The nicotine concentrations are expressed as nicotine base. Minipumps administered nicotine or saline solution at a rate of approximately 0.48 μl/hr. Dosages were calculated based on body weight such that nicotine animals received either 6 mg/kg/day or 12 mg/kg/day depending on experimental group assignment. These dosages have been used extensively in studies with rats that have been replicated in experiments with human smokers (Grunberg et al., 1984; Grunberg, 1986).

Identical surgical implant procedures were followed as in **Experiment 1**.

Stress Manipulation

Animals in the stress condition were restrained in commercially available finger-like restraining devices (Centrap Cage, Fisher Scientific) 20 min/day beginning the day after surgery. Subjects were placed in the Centrap cage and the restraining "fingers" were tightened until subjects were immobilized, but not pinched or in pain. Restrained animals

were checked every 5 min during the stress procedure to insure the manipulation did not result in pain or undue distress. This restraint procedure has reliably produced elevations in hormones associated with a stress response, including adrenocorticotropin hormone (ACTH) and corticosterone (Kant et al., 1983; Flores et al., 1990; Acri, 1992, 1994; Raygada et al., 1992; Klein, 1997).

Procedure

Baseline Phase. Table 4 presents the timeline of Experiment 2. Subjects were gentled once each day for three days. Subjects then underwent two ASR-PPI acclimation exposures and a baseline ASR-PPI measure. For the first ASR-PPI acclimation, subjects were placed in the open-air cages inside the test chamber but not exposed to the noise stimuli. For the second ASR-PPI acclimation, subjects were placed in the apparatus and exposed to the noise stimuli. Three days after the second acclimation exposure ASR-PPI baseline responses were measured. These data are the reported ASR-PPI baseline values. Throughout the Baseline Phase subjects' body weights were measured every other day. The Baseline Phase lasted approximately two weeks.

During Drug Administration Phase. After the completion of baseline measures, subjects were assigned within sex and strain to drug (0 mg/kg/day, 6 mg/kg/day, or 12 mg/kg/day nicotine) and stress (stress or no stress) groups in a manner insuring comparable initial body weights. This assignment resulted in 24 balanced groups of 10 subjects per group (6 groups each of Sprague-Dawley males, Sprague-Dawley females, Long-Evans males, and Long-Evans females). Minipumps containing the appropriate solutions were implanted as described in Drug Administration and Surgical Procedure on

During Drug Administration day 1. On During Drug Administration day 2 subjects in the stress condition began undergoing 20 min/day of restraint stress. These subjects were stressed every day for the remainder of the Experimental phase.

ASR and PPI were measured for all subjects on During Drug day 2 (after 24 hours of nicotine or saline administration and one day of stress manipulation), on During Drug day 6 (after 5 days of drug administration and stress manipulation), and on During Drug day 12 (after 11 days of drug administration and stress manipulation). Subjects in the stress condition were stressed approximately 30 min before the ASR-PPI measures. This procedure has resulted in stress-related changes in ASR-PPI (Acri, 1992, 1994). Body weights were measured every other day during the During Drug Administration Phase. Subjects were sacrificed without anesthesia on During Drug day 15.

Results

Data Analytic Strategy

Three subjects were dropped from all analyses: one Sprague-Dawley male (12 mg/kg/day-Stress group) and two Sprague-Dawley females (one from the 12 mg/kg/day-No stress group and one from the 6 mg/kg/day-Stress group). In each case surgical complications resulted in failure of the minipump to deliver drug reliably. This failure was evident because these subjects did not lose weight and the site of the minipumps appeared encapsulated or infected.

Body Weight. Body weight data were analyzed by repeated-measures analysis of variance (ANOVA). Separate analyses were conducted for males and females of each strain with time as the within-subject factor, and drug and stress as between-subjects factors. Males and females were analyzed separately because significant body weight differences existed between the sexes at all time points and empirical literature indicates that effects of nicotine on body weight are greater in females than in males. Strains were analyzed separately because trends toward strain differences in body weight were noted at several time points. Analyses included six time points: Baseline Day 14, and During Drug Phase Days 1, 4, 6, 12, and 14. Subsequent ANOVAs with factors of drug and stress condition were conducted at each time point to determine which groups differed significantly. Post hoc tests were conducted when necessary to determine which drug groups differed.

ASR-PPI. Startle amplitudes to each stimulus (98, 112, and 122dB) were calculated as in **Experiment 1** by subtracting the amount of platform displacement in g on

the no stimulus trials (body weight) from the amount of platform displacement in response to each stimulus for each subject at each time point. The remainders were analyzed as startle amplitude. Amount of pre-pulse inhibition was calculated by subtracting the amount of platform displacement in g on no stimulus trials from each stimulus (98, 112, and 122dB) when presented with pre-pulse. This amount, representing startle amplitude to each stimulus with pre-pulse, was then subtracted from the startle amplitude to each stimulus (98, 112, and 122dB) without pre-pulse. This calculation was done for each subject at each time point. The remainders were analyzed as amount of pre-pulse inhibition. Percent pre-pulse inhibition was calculated by expressing amount of pre-pulse to each stimulus (98, 112, and 122dB) with pre-pulse as a percentage of startle amplitude. Specifically, amount of pre-pulse for each stimulus was multiplied times 100 and divided by startle amplitude to that stimulus without pre-pulse. This calculation was done for each subject at each time point. The products were analyzed as percent pre-pulse inhibition.

In order to minimize the chances of spurious findings because of the number of tests run on ASR and PPI responses, three strategies were employed. First, an initial MANOVA was performed that included startle amplitudes (98dB, 112dB, and 122dB) without pre-pulse and startle amplitudes (98dB, 112dB, and 122dB) with pre-pulse from the Baseline Phase and During Drug Phase day 6 to determine whether stimulus intensity and stimulus type (i.e., pre-pulse or no pre-pulse) significantly affected responses. This analysis indicated that responses were significantly different to each stimulus intensity and stimulus type with the exception of Day 6 responses to the 98dB stimulus with pre-pulse. Because responses to the majority of the stimuli with and without pre-pulse differed, each

stimulus type and intensity was analyzed separately.

Second, the alpha level for new, non-replicating findings was reduced to 0.01. These findings are designated in the text that follows with the notation "$p < 0.01$." Where findings fell between the $p < 0.01$ and $p < 0.05$ cut-offs, they are reported with specific p values, e.g., "$p = 0.036$." Third, for findings that replicated Experiment 1 and other empirical work cited, an alpha level of 0.05 was used. This alpha level was chosen because of the multiplicative nature of probabilities. That is, because the alpha level for Experiment 1 and work cited in the Introduction was 0.05, any replicating finding in Experiment 2 that also made the 0.05 criteria actually had a probability of Type I error of 0.0025. Replicating findings, therefore, are designated in the text that follows by "$p \leq 0.05$." The **Discussion** section emphasizes replicating findings.

Three-way analyses of covariance (ANCOVAs) were performed on ASR amplitudes to each stimulus (98dB, 112dB, and 122dB) with strain, sex, drug, and stress as separate factors on During Drug Administration Days 2, 6, and 12 for all subjects using baseline responses as covariates. Similar analyses were performed on amount of pre-pulse inhibition and percent pre-pulse inhibition in response to each stimulus (98dB, 112dB, and 122dB) with pre-pulse on Days 2, 6, and 12 using baseline responses as covariates. Baseline covariates were used because preliminary analyses indicated significant baseline differences among some but not all subgroups.

In addition, ANCOVAs were conducted at each time point on each strain separately because of *a priori* hypotheses that strains would differ in responses to nicotine. ANCOVAs also were conducted on males and females separately (collapsing across

strain) at each time point because of *a priori* hypotheses that females would be more sensitive to the effects of nicotine than males. Also, separate ANCOVAs were conducted on Day 2, 6, and 12 responses of within strain-within sex subgroups (e.g., Sprague-Dawley males, Sprague-Dawley females, Long-Evans males, Long-Evans) separately because of *a priori* hypotheses that sex and strain would affect responses to nicotine. Where necessary (e.g., for drug effects) Tukey's HSD post hoc tests were used unless otherwise noted. One-way ANOVAs were used for specific hypothesis-testing (e.g., for planned comparisons).

Results: Body Weight

Figure 15 presents body weights in grams of male and female Sprague-Dawley and Long-Evans subjects at multiple time points. Findings reported below are significant at the $p < 0.05$ level unless otherwise noted.

Nicotine administration decreased body weights of all subjects regardless of strain or sex {Sprague-Dawley males [F (2, 52) = 3.394], Sprague-Dawley females [F (2, 52) = 4.380], Long-Evans females [F (2, 54) = 7.629], and Long-Evans males [F (2, 54) = 2.778, $p = 0.071$]}. Tukey's HSD post hoc tests indicated that: for Sprague-Dawley males and Sprague-Dawley females 12/mg/kg/day nicotine-treated subjects weighed less than saline-treated subjects; for Long-Evans females 12 mg/kg/day nicotine-treated subjects weighed less than saline-treated subjects and 6 mg/kg/day nicotine-treated subjects; and for Long-Evans males there was a trend toward ($p = 0.060$) the 12 mg/kg/day nicotine-treated subjects weighing less than the saline-treated subjects. Stress also decreased body weights of Sprague-Dawley males [F (1, 52) = 4.040], and Long-

Evans males [F (1, 54) = 4.432], and tended to decrease body weights of Long-Evans females [F (1, 54) = 3.462, p = 0.068]. In addition, nicotine administration decreased body weights of Long-Evans females in a dose-response fashion but stress decreased saline-treated subjects' body weights only [F (2, 54) = 3.976].

All subjects' body weights increased over time {Sprague-Dawley males [F (5, 260) = 270.800], Sprague-Dawley females [F (5, 260) = 63.732], Long-Evans males [F (5, 270) = 155.511], and Long-Evans females [F (5, 270) = 27.505]}. Nicotine administration and stress also altered subjects' body weights over time. Specifically, 12 mg/kg/day nicotine-treated subjects gained weight more slowly over time than 6 mg/kg/day nicotine-treated animals, and 6 mg/kg/day nicotine-treated subjects gained weight more slowly over time than saline-treated subjects regardless of strain or sex {Sprague-Dawley males [F (10, 260) = 13.677], Sprague-Dawley females [F (10, 260) = 8.019], Long-Evans males [F (10, 270) = 8.433], and Long-Evans females [F (10, 270) = 3.743]}. Stress altered body weights of males only over time. Specifically, stressed males gained weight more slowly over time than non-stressed males {Sprague-Dawley males [F (5, 260) = 18.320], Long-Evans males [F (5, 270) = 12.758]}.

Body weight reductions as a result of drug also were evident on individual measurement days. Nicotine administration reduced body weights of Sprague-Dawley males on Day 4 [F (2, 52) = 4.707], Day 6 [F (2, 52) = 6.040], Day 12 [F (2, 52) = 5.881] and Day 14 [F (2, 52) = 7.994]; of Sprague-Dawley females on Day 4 [F (2, 52) = 5.742], Day 6 [F (2, 52) = 8.124], Day 12 [F (2, 52) = 7.055], and Day 14 [F (2, 52) = 8.568]; of Long-Evans males on Day 6 [F (2, 54) = 5.859], Day 12 [F (2, 54) = 5.008], and Day 14

[F (2, 54) = 5.516]; and of Long-Evans females on Day 4 [F (2, 54) = 14.086], Day 6 [F (2, 54) = 5.444], Day 12 [F (2, 54) = 3.634], and Day 14 [F (2, 54) = 13.159]. Tukey's HSD post hoc tests indicated that 12 mg/kg/day nicotine-treated subjects weighed less than saline-treated subjects at all time points with the exception of Long-Evans males on During Drug Day 4 and Long-Evans females on During Drug Day 12. In addition, on Day 4 Sprague-Dawley and Long-Evans female 6 mg/kg/day subjects weighed less than saline-treated subjects and Long-Evans female 12 mg/kg/day subjects weighed less than the 6 mg/kg/day nicotine group.

Stress reduced body weights of Sprague-Dawley males on Day 6 [F (1, 52) = 4.153], Day 12 [F (1, 52) = 10.804], and Day 14 [F (1, 52) = 11.131]; of Long-Evans males on Day 12 [F (1, 54) = 8.568] and Day 14 [F (1, 54) = 12.014]; and of Long-Evans females on Day 12 [F (1, 54) = 5.881] and Day 14 [F (1, 54) = 6.436]. For Long-Evans females nicotine decreased body weight in non-stressed subjects in a dose-response fashion and stress decreased body weight in saline-treated subjects only on Day 4 [F (2, 54) = 3.767] and Day 14 [F (2, 54) = 4.558].

Results: Acoustic Startle and Pre-Pulse Inhibition

Startle Amplitude to 98dB. Figure 16 presents startle amplitude in g to the 98dB stimulus without pre-pulse on During Drug Days 2, 6, and 12, respectively. On **Day 2** nicotine administration increased startle amplitudes in Sprague-Dawley subjects but decreased startle amplitudes in Long-Evans subjects [F (2, 212) = 4.308, $p < 0.05$]. In female subjects nicotine increased startle in a dose-response fashion for Sprague-Dawley females but decreased startle for Long-Evans females [F (2, 105) = 12.528, $p < 0.05$]. For

Long-Evans subjects 6 mg/kg/day and 12 mg/kg/day nicotine administration decreased startle [F (2, 107) = 8.148, $p < 0.05$]. For Sprague-Dawley females nicotine-induced startle increases [F (2, 51) = 7.389, $p < 0.05$] were evident in the 12 mg/kg/day group with the 6 mg/kg/day group also tending ($p = 0.063$) to exhibit the same pattern. For Long-Evans females nicotine-induced startle decreases [F (2, 53) = 6.964, $p < 0.05$] were clear in both the 6 mg/kg/day and 12 mg/kg/day nicotine groups.

Sprague-Dawley subjects startled more than did Long-Evans subjects [F (1, 212) = 5.032, $p < 0.05$]. Stress increased startle amplitudes in Sprague-Dawley males and Long-Evans females but decreased amplitudes in Sprague-Dawley females and Long-Evans males [F (1, 212) = 4.432, $p = 0.036$]. For Sprague-Dawley subjects stress tended to increase male startle amplitudes but decrease female amplitudes [F (1, 104) = 3.760, $p = 0.055$]. Long-Evans females tended to startle more than Long-Evans males [F (1, 107) = 3.682, $p = 0.058$].

On **Day 6** nicotine increased startle amplitudes in Sprague-Dawley subjects but decreased Long-Evans' amplitudes [F (2, 212) = 3.524, $p < 0.05$]. Nicotine administration also tended to increase male Sprague-Dawley startle amplitudes but decrease male Long-Evans amplitudes [F (2, 106) = 2.843, $p = 0.063$], and to increase startle amplitudes for Sprague-Dawley subjects [F (2, 104) = 2.794, $p = 0.066$]. Females startled more than did males [F (1, 212) = 5.471, $p = 0.02$], especially among Long-Evans subjects [F (1, 107) = 19.004, $p < 0.01$]. Sprague-Dawley subjects startled more than did Long-Evans subjects [F (1, 212) = 10.431, $p < 0.01$], especially among males [F (1, 106) = 13.497, $p < 0.05$]. Sprague-Dawley males startled more than Sprague-Dawley females

and Long-Evans females startled more than Long-Evans males [F (1, 212) = 6.433, p = 0.01].

On **Day 12** nicotine tended to increase Sprague-Dawley subjects' startle amplitudes and decrease Long-Evans subjects' amplitudes [F (2, 212) = 2.682, p = 0.071]. This pattern was clear in male subjects [F (2, 106) = 5.949, p < 0.05]. Nicotine-induced increases in startle also were evident in Sprague-Dawley subjects [F (2, 104) = 3.072, p = 0.05], especially Sprague-Dawley males [F (2, 52) = 7.253, p < 0.05]. Specifically, the 12 mg/kg/day group startled more than the 6 mg/kg/day and saline groups. For Sprague-Dawley subjects 12 mg/kg/day nicotine increased male startle amplitudes but both nicotine dosages decreased startle amplitudes in females [F (2, 107) = 3.161, p = 0.047]. Considering all subjects, the effects of nicotine depended on strain and sex: 12 mg/kg/day nicotine increased Sprague-Dawley male amplitudes, 6 mg/kg/day and 12 mg/kg/day decreased Sprague-Dawley female amplitudes, 6 mg/kg/day increased Long-Evans male amplitudes but 12 mg/kg/day decreased amplitudes, and 6 mg/kg/day and 12 mg/kg/day increased Long-Evans female startle amplitudes [F (2, 212) = 3.556, p = 0.03]. Females startled more than did males [F (1, 212) = 11.780, p < 0.01], especially among Long-Evans subjects [F (1, 107) = 8.442, p < 0.01].

Startle Amplitude to 112dB. Figure 17 presents startle amplitude in g to the 112dB stimulus without pre-pulse on Experimental Days 2, 6, and 12, respectively. On **Day 2** nicotine administration increased startle amplitudes in Sprague-Dawley subjects but decreased Long-Evans' amplitudes [F (2, 212) = 4.522, p < 0.05], especially among females [F (2, 105) = 9.393, p < 0.05]. Nicotine tended to decrease male Sprague-Dawley

amplitudes but increase female Sprague-Dawley amplitudes [F (2, 104) = 2.931, p = 0.058]. The nicotine-induced increase for Sprague-Dawley females was clear when just these subjects were examined [F (2, 51) = 4.015, $p < 0.05$] with the 12 mg/kg/day group startling more than the saline group. Nicotine administration decreased amplitudes of Long-Evans subjects [F (2, 107) = 9.168, $p < 0.05$], especially 6 mg/kg/day nicotine with the 12 mg/kg/day dosage also tending to decrease startle (p = 0.074). This pattern was clear among Long-Evans males [F (2, 53) = 3.226, $p < 0.05$] and among Long-Evans females [F (2, 53) = 7.533, $p < 0.05$]. In each case, the 6 mg/kg/day nicotine group startled significantly less than the saline group. Sprague-Dawley subjects startled more than did Long-Evans subjects [F (1, 212) = 12.608, $p < 0.01$] whether subjects were male [F (1, 106) = 5.657, $p < 0.05$] or female [F (1, 105) = 7.968, $p < 0.01$].

On **Day 6** nicotine administration increased Sprague-Dawley subjects' amplitudes but decreased Long-Evans subjects' amplitudes [F (2, 212) = 7.092, $p < 0.05$], among males [F (2, 106) = 3.185, $p < 0.05$] and also among females [F (2, 105) = 4.227, $p < 0.05$]. The nicotine-induced increase was evident among Sprague-Dawley subjects [F (2, 104) = 4.515, $p < 0.05$], with the 12 mg/kg/day group startling more than the saline group. This pattern also was clear among Sprague-Dawley females [F (2, 51) = 5.198, $p < 0.05$] with the 12 mg/kg/day group startling more than the saline group and the 6 mg/kg/day group tending to startle more (p = 0.064) than the saline group. For Long-Evans subjects nicotine tended to reduce startle [F (2, 107) = 2.937, p = 0.057], with the 6 mg/kg/day nicotine group tending to startle less than the saline group. This pattern was evident among Long-Evans males [F (2, 53) = 4.012, $p < 0.05$] with the 6 mg/kg/day

group startling less than the saline group. Sprague-Dawley subjects startled more than did Long-Evans subjects [F (1, 212) = 19.725, $p < 0.01$] whether subjects were male [F (1, 106) = 13.031, $p < 0.05$] or female [F (1, 105) = 7.785, $p < 0.01$]. Female Long-Evans subjects startled more than did male Long-Evans subjects [F (1, 107) = 5.280, $p = 0.024$].

On **Day 12** nicotine increased startle amplitudes of Sprague-Dawley males but decreased amplitudes of Long-Evans males [F (2, 106) = 3.341, $p < 0.05$]. The increase for Sprague-Dawley males was clear [F (2, 52) = 4.357, $p < 0.05$] with the 12 mg/kg/day group startling more than the saline group. Long-Evans females startled more than did Long-Evans males [F (1, 107) = 3.988, $p = 0.048$].

Startle Amplitude to 122dB. Figure 18 presents startle amplitude in g to the 122dB stimulus without pre-pulse on Experimental Days 2, 6, and 12. On **Day 2** nicotine tended to increase Sprague-Dawley startle amplitudes but decrease Long-Evans amplitudes [F (1, 212) = 2.764, $p = 0.065$]. This pattern was clear among females [F (2, 105) = 5.339, $p < 0.01$] with nicotine increasing startle amplitudes in Sprague-Dawley females but having no effect in Long-Evans females. Nicotine decreased startle amplitudes in males but increased amplitudes in females [F (1, 212) = 4.879, $p < 0.01$], especially among Sprague-Dawley subjects [F (2, 104) = 3.390, $p = 0.037$]. This increase for females [F (2, 105) = 6.731, $p < 0.01$] consisted of the 12 mg/kg/day nicotine group startling more than the saline group, and was specific to Sprague-Dawley females [F (2, 51) = 7.585, $p < 0.05$]. Stress and nicotine administration together tended to affect Long-Evans males differently [F (2, 53) = 2.886, $p = 0.065$] with nicotine increasing startle amplitudes for non-stressed subjects and decreasing amplitudes for stressed subjects.

Overall, stress increased startle amplitudes for males but decreased amplitudes for females [F (1, 211) = 4.449, p = 0.036]. This pattern was clear among Long-Evans females where stressed subjects startled less than did non-stressed subjects [F (1, 53) = 4.938, p = 0.03]. Males startled more than did females [F (1, 212) = 4.766, p = 0.03], especially among Long-Evans subjects [F (2, 107) = 4.315, p < 0.05]. Sprague-Dawley subjects startled more than did Long-Evans subjects [F (1, 212) = 33.709, p < 0.01] whether subjects were male [F (1, 106) = 12.309, p < 0.05] or female [F (1, 105) = 24.593, p < 0.01].

On **Day 6** nicotine increased startle amplitudes in Sprague-Dawley subjects but decreased amplitudes in Long-Evans subjects [F (2, 212) = 10.307, p < 0.05] whether subjects were male [F (2, 106) = 5.075, p < 0.05] or female [F (2, 105) = 5.486, p < 0.05]. The nicotine-induced startle increase was evident overall [F (2, 212) = 3.299, p = 0.039] with the 12 mg/kg/day group startling more than the saline group and more than the 6 mg/kg/day group (Fisher's LSD). This increase also was clear among Sprague-Dawley subjects [F (2, 104) = 7.107, p < 0.05], among female subjects [F (2, 105) = 5.805, p < 0.01], and among Sprague-Dawley female subjects [F (2, 51) = 7.681, p < 0.05]. In all cases the 12 mg/kg/day group startled more than the saline group. For Long-Evans subjects nicotine decreased startle [F (2, 107) = 5.238, p < 0.05], especially in the 6 mg/kg/day group. This pattern was evident for Long-Evans males [F (2, 53) = 13.217, p < 0.05] with both the 6 mg/kg/day and 12 mg/kg/day groups startling less than the saline group. Among all subjects nicotine increased startle amplitudes in females but decreased amplitudes in males [F (2, 212) = 4.494, p = 0.01], especially among Long-Evans subjects

[F (2, 107) = 6.072, p < 0.01]. For males stress tended to increase startle [F (1, 106) = 3.295, p = 0.072], especially for Sprague-Dawley males [F (1, 52) = 3.402, p = 0.071]. Sprague-Dawley subjects startled more than did Long-Evans subjects [F (1, 212) = 52.800, p < 0.01], whether subjects were male [F (1, 106) = 26.630, p < 0.05] or female [F (1, 105) = 25.551, p < 0.01].

On **Day 12** nicotine increased startle amplitude in Sprague-Dawley subjects but had no effect in Long-Evans subjects [F (2, 212) = 3.084, p < 0.05]. The nicotine-induced increase was evident overall [F (2, 212) = 7.972, p < 0.01] as well as among Sprague-Dawley subjects [F (2, 104) = 6.511, p < 0.05], among male subjects [F (2, 106) = 7.180, p < 0.01], and among Sprague-Dawley male subjects [F (2, 52) = 5.785, p < 0.05]. In all cases, the 12 mg/kg/day nicotine group startled more than the saline group. Sprague-Dawley subjects startled more than did Long-Evans subjects [F (1, 212) = 13.600, p < 0.01] whether subjects were male [F (1, 106) = 8.061, p < 0.05] or female [F (1, 105) = 5.153, p = 0.025].

Amount Pre-Pulse Inhibition to 98dB w/ Pre-Pulse. Figure 19 presents amount of pre-pulse inhibition in g to the 98dB stimulus with pre-pulse on Experimental Days 2, 6, and 12. On **Day 2** nicotine increased PPI amounts in Sprague-Dawley females but decreased PPI amounts in Long-Evans females [F (2, 105) = 4.127, p < 0.05]. This increase was evident among Sprague-Dawley females [F (2, 51) = 2.935, p = 0.062]. The effects of stress on PPI depended on the strain and sex of subjects [F (1, 212) = 4.160, p = 0.043] with stress increasing PPI amounts in Sprague-Dawley males and Long-Evans females, but decreasing PPI amounts in Sprague-Dawley females and Long-Evans males.

Increased PPI in males but decreased PPI in females as a result of stress also was evident in Sprague-Dawley subjects [F (1, 104) = 4.359, p = 0.039]. Sprague-Dawley subjects exhibited greater pre-pulse inhibition (PPI) amounts than Long-Evans subjects [F (1, 212) = 3.882, p = 0.05].

On **Day 6** nicotine tended to increase Sprague-Dawley male PPI amounts but decrease Long-Evans male PPI amounts [F (2, 106) = 3.029, p = 0.053]. Sprague-Dawley subjects exhibited greater PPI amounts than Long-Evans subjects [F (1, 212) = 8.106, p < 0.01], especially among male subjects [F (1, 106) = 8.591, p < 0.05]. Males exhibited greater PPI amounts than females [F (1, 212) = 8.308, p < 0.01], especially among Sprague-Dawleys [F (1, 104) = 8.406, p < 0.05]. Although Sprague-Dawley males exhibited greater PPI amounts than Sprague-Dawley females, Long-Evans females exhibited greater PPI amounts than Long-Evans males [F (1, 212) = 4.728, p = 0.03].

On **Day 12** nicotine tended to increase PPI amounts for Sprague-Dawley males [F (2, 52) = 3.000, p = 0.058]. Sprague-Dawley females exhibited greater PPI amounts than Long-Evans females [F (1, 105) = 4.491, p = 0.036].

Amount Pre-Pulse Inhibition to 112dB w/ Pre-Pulse. Figure 20 presents amount of pre-pulse inhibition in g to the 112dB stimulus with pre-pulse on Experimental Days 2, 6, and 12, respectively. On **Day 2** nicotine decreased PPI amounts [F (2, 212) = 3.610, p = 0.029], with the 6 mg/kg/day and 12 mg/kg/day groups exhibiting less pre-pulse inhibition than the saline group (Fisher's LSD). This pattern was evident in Long-Evans subjects [F (2, 107) = 5.349, p < 0.05], in which the 6 mg/kg/day nicotine group exhibited less PPI than the saline group. For males nicotine also tended to decrease PPI

[F (2, 106) = 2.627, p = 0.077], especially in the 6 mg/kg/day group. Among Long-Evans females nicotine decreased PPI [F (2, 53) = 3.166, p = 0.05] regardless of dose. Sprague-Dawley subjects exhibited greater PPI amounts than Long-Evans subjects [F (1, 212) = 4.615, p = 0.033] and males had greater PPI than females [F (1, 212) = 10.550, p < 0.01] among Sprague-Dawley [F (1, 104) = 5.387, p = 0.022] and Long-Evans subjects [F (1, 107) = 5.258, p < 0.05].

On **Day 6** nicotine increased PPI amounts in Sprague-Dawley subjects but decreased PPI amounts in Long-Evans subjects [F (2, 212) = 4.800, p < 0.05], especially in female subjects [F (2, 105) = 4.328, p < 0.05]. Nicotine-induced PPI increases were evident for Sprague-Dawley subjects [F (2, 104) = 3.296, p < 0.05], especially in Sprague-Dawley female responses [F (2, 51) = 3.817, p < 0.05]. In each case the 12 mg/kg/day nicotine group exhibited greater PPI than the saline group. Sprague-Dawley subjects exhibited greater PPI amounts than Long-Evans subjects [F (1, 212) = 33.730, p < 0.01] whether subjects were male [F (1, 106) = 13.991, p < 0.05] or female [F (1, 105) = 20.453, p < 0.01].

On **Day 12** for males the effects of nicotine depended on strain and stress [F (2, 106) = 3.512, p = 0.033]. Specifically, nicotine increased PPI in a dose-response fashion for non-stressed Sprague-Dawley males but nicotine + stress produced an inverted-U pattern with PPI amounts in saline and 6 mg/kg/day subjects increased to a greater extent than nicotine alone, and 12 mg/kg/day group responses similar to saline, non-stress responses. For non-stressed Long-Evans males nicotine's effects also followed an inverted U-shaped pattern similar to that for stressed Sprague-Dawley males but with

changes of smaller magnitude. For stressed Long-Evans males PPI amounts for saline and 6 mg/kg/day group were approximately equal but were decreased by the 12 mg/kg/day dose. This pattern also was clear in Sprague-Dawley males [F (2, 52) = 3.879, p = 0.027] with nicotine's effects in the non-stress group occurring in a dose-response fashion but following an inverted U-shaped pattern in the stressed group.

Stress decreased PPI amounts for Sprague-Dawley subjects but increased PPI amounts for Long-Evans subjects [F (1, 212) = 6.585, p = 0.01], especially among female subjects [F (1, 105) = 5.052, p = 0.027]. The PPI increase also was evident among Long-Evans subjects regardless of sex [F (1, 107) = 12.660, p < 0.01] as well as among Long-Evans males [F (1, 53) = 3.468, p = 0.068] and females [F (1, 53) = 4.443, p < 0.01]. Sprague-Dawley subjects exhibited greater PPI amounts than Long-Evans subjects [F (1, 212) = 20.501, p < 0.01] among male [F (1, 106) = 8.735, p < 0.05] and female subjects [F (1, 105) = 12.747, p < 0.01].

Amount Pre-Pulse Inhibition to 122dB w/ Pre-Pulse. Figure 21 presents amount of pre-pulse inhibition in g to the 122dB stimulus with pre-pulse on Experimental Days 2, 6, and 12, respectively. On **Day 2** for females nicotine increased PPI [F (2, 105) = 3.752, p = 0.027], with the 12 mg/kg/day nicotine group exhibiting greater pre-pulse amounts than the saline group (Fisher's LSD). Nicotine decreased PPI, however, in Long-Evans males [F (2, 53) = 4.802, p < 0.05] and Long-Evans females [F (2, 53) = 4.198, p < 0.05], especially the 12 mg/kg/day dosage.

In general stress increased male PPI amounts but decreased female PPI amounts [F (1, 212) = 4.370, p = 0.038]. Nicotine's effects also depended on stress [F (2, 212) =

5.192, $p < 0.01$]. Specifically, nicotine's effects in the non-stressed group followed a U-shaped function with 6 mg/kg/day nicotine decreasing PPI amounts below saline group levels and 12 mg/kg/day nicotine increasing PPI amounts to slightly more than saline group levels. For stressed subjects nicotine's effects followed an inverted U-shaped pattern with 6 mg/kg/day nicotine increasing PPI amounts over saline control levels but 12 mg/kg/day nicotine decreasing PPI amounts below saline control levels. Nicotine increased female PPI amounts in a dose-response fashion but decreased male PPI responses in a dose-response pattern [$\underline{F}$ (2, 212) = 5.316, $p < 0.01$].

For Long-Evans subjects stressed and non-stressed saline and 6 mg/kg/day groups exhibited similar responses but 12 mg/kg/day nicotine increased responses in the absence of stress and decreased responses when combined with stress [$\underline{F}$ (2, 107) = 3.733, $p = 0.027$]. In addition, Long-Evans males exhibited greater PPI amounts than females at the 6 mg/kg/day dose but males and females exhibited similar responses at the 12 mg/kg/day dose [$\underline{F}$ (2, 107) = 9.041, $p < 0.01$]. For males stress increased responses in the saline and 6 mg/kg/day groups above non-stressed subjects' responses but decreased responses in the 12 mg/kg/day group below non-stressed subjects responses [$\underline{F}$ (2, 106) = 3.624, $p = 0.03$]. Sprague-Dawley subjects exhibited greater PPI amounts than Long-Evans subjects [$\underline{F}$ (1, 212) =31.835, $p < 0.01$] among males [$\underline{F}$ (1, 106) = 12.904, $p < 0.05$] and females [$\underline{F}$ (1, 105) =20.495, $p < 0.01$]. Males exhibited greater PPI than females [$\underline{F}$ (1, 212) = 14.478, $p < 0.01$] among Sprague-Dawley [$\underline{F}$ (1, 104) = 5.505, $p < 0.02$] and Long-Evans subjects [$\underline{F}$ (1, 107) = 12.716, $p < 0.05$].

On **Day 6** nicotine increased PPI amounts in Sprague-Dawley subjects but

decreased PPI amounts in Long-Evans subjects [F (2, 212) = 5.291, $p < 0.05$], especially among females [F (2, 105) = 4.290, $p < 0.05$]. The nicotine-induced PPI increase was evident in Sprague-Dawley subjects [F (2, 104) = 4.416, $p < 0.05$] with the 12 mg/kg/day group exhibiting greater pre-pulse amounts than the saline group. This pattern also was evident among females [F (2, 105) = 4.564, $p = 0.01$], especially Sprague-Dawley females [F (2, 51) = 5.298, $p < 0.05$]. The nicotine-induced PPI decrease was clear among Long-Evans males [F (2, 53) = 4.088, $p < 0.05$] where the 6 mg/kg/day group exhibited less PPI than the saline group. Nicotine increased PPI amounts in females but decreased PPI in males [F (2, 212) = 3.677, $p = 0.027$]. In Long-Evans subjects nicotine greatly decreased PPI amounts in males and decreased PPI amounts in females to a lesser extent [F (2, 107) = 4.042, $p = 0.02$].

For males stress increased Sprague-Dawley PPI amounts but decreased Long-Evans PPI amounts [F (1, 106) = 4.243, $p = 0.042$]. Stressed Sprague-Dawley males exhibited greater PPI amounts than non-stressed males [F (1, 52) = 5.288, $p < 0.05$]. Sprague-Dawley subjects exhibited greater PPI amounts than Long-Evans subjects [F (1, 212) = 89.320, $p < 0.01$] among males [F (1, 106) = 35.648, $p < 0.05$] and females [F (1, 105) = 52.786, $p < 0.01$]. Males had greater PPI than females [F (1, 212) = 6.569, $p = 0.01$], especially among Long-Evans subjects [F (1, 107) = 6.790, $p < 0.05$].

On **Day 12** nicotine increased PPI amounts [F (2, 212) = 4.926, $p < 0.01$], especially the 12 mg/kg/day dosage, especially in males [F (2, 106) = 3.468, $p = 0.035$], and in Sprague-Dawley subjects [F (2, 104) = 2.782, $p = 0.067$]. For Long-Evans subjects nicotine increased PPI amounts in non-stressed subjects and decreased PPI

amounts in stressed subjects [F (2, 107) = 3.616, p = 0.03]. For females stress increased Sprague-Dawley subjects' PPI amounts but decreased Long-Evans subjects' PPI amounts [F (1, 105) = 5.207, p = 0.025]. For Sprague-Dawley females stress increased PPI [F (1, 51) = 5.344, p = 0.025]. Sprague-Dawley subjects exhibited greater PPI amounts than Long-Evans subjects [F (1, 212) = 39.660, p < 0.01] among males [F (1, 106) = 14.406, p < 0.05] and females [F (1, 105) = 26.069, p < 0.01]. Males tended to exhibit greater PPI amounts than females [F (1, 212) = 3.793, p = 0.053].

Percent Pre-Pulse Inhibition to 98dB w/ Pre-Pulse. Figure 22 presents percent pre-pulse inhibition (PPP) to the 98dB stimulus with pre-pulse on Experimental Days 2, 6, and 12, respectively. **Day 2** analyses revealed no significant findings. On **Day 6** nicotine increased PPP in males in a dose-response pattern and affected female responses in an inverted U-shaped pattern, with peak responses at the 6 mg/kg/day dose [F (2, 207) = 3.878, p = 0.022]. Nicotine administration increased PPP of Long-Evans subjects [F (2, 101) = 3.263, p = 0.042] and of male subjects [F (2, 99) = 3.707, p = 0.028] with the 12 mg/kg/day group exhibiting greater PPP than the saline group in both cases. Males exhibited greater PPP than females [F (1, 207) = 25.956, p < 0.01] among Sprague-Dawley [F (1, 103) = 26.727, p < 0.01] and Long-Evans subjects [F (1, 101) = 7.455, p < 0.05]. On **Day 12** Sprague-Dawley subjects exhibited greater PPP than Long-Evans subjects [F (1, 207) = 4.558, p = 0.034], especially among females [F (1, 105) = 10.433, p < 0.01]. Males exhibited more PPP than females [F (1, 207) = 4.909, p = 0.028].

Percent Pre-Pulse Inhibition to 112dB w/ Pre-Pulse. Figure 23 presents percent pre-pulse inhibition (PPP) to the 112dB stimulus with pre-pulse on Experimental

Days 2, 6, and 12, respectively. On **Day 2** nicotine (6 and 12 mg/kg/day) decreased PPP for Sprague-Dawley subjects but for Long-Evans subjects 6 mg/kg/day nicotine increased PPP and 12 mg/kg/day nicotine decreased PPP [F (2, 213) = 4.486, p = 0.01]. Overall nicotine decreased PPP [F (2, 213) = 3.345, p = 0.037] with the 6 mg/kg/day and 12 mg/kg/day groups exhibiting less PPP than the saline group (Fisher's LSD). Nicotine's PPP-reducing effects also were evident in Sprague-Dawley subjects [F (2, 104) = 4.998, p < 0.01] and in Sprague-Dawley males [F (2, 52) = 4.500, p = 0.016]. In each case the 6 mg/kg/day group had lower PPP responses than the saline group. In contrast, for Long-Evans males [F (2, 52) = 3.250, p = 0.047] the 6 mg/kg/day group tended to exhibit more PPP than the 12 mg/kg/day group (p=0.056). Males exhibited greater PPP responses than females [F (1, 213) = 12.950, p < 0.01], especially among Long-Evans subjects [F (1, 106) = 18.287, p < 0.05]. On **Day 6** Sprague-Dawley subjects exhibited greater PPP than Long-Evans subjects [F (1, 213) = 11.542, p < 0.01], especially among females [F (1, 105) = 17.078, p < 0.01].

On **Day 12** nicotine decreased PPP for Sprague-Dawley subjects [F (2, 104) = 3.997, p = 0.02] and for females [F (2, 105) = 3.083, p = 0.05] and tended to decrease PPP of Sprague-Dawley males [F (2, 52) = 2.970, p = < 0.060]. In each case the 12 mg/kg/day nicotine group exhibited less PPP than the saline group. Sprague-Dawley subjects exhibited greater PPP than Long-Evans subjects [F (1, 214) = 14.921, p < 0.01] among males [F (1, 106) = 3.868, p < 0.05] and females [F (1, 105) = 15.641, p < 0.01]. Stress decreased Sprague-Dawley subjects' responses but increased Long-Evans subjects' responses [F (1, 214) = 6.213, p = 0.01], especially among females [F (1, 105) = 8.932, p

< 0.01]. The stress-induced increase was clear for Long-Evans subjects [F (1, 107) = 4.410, p = 0.038] and for Long-Evans females [F (1, 53) = 8.341, p < 0.01]. The tendency toward a stress-induced decrease was evident in Sprague-Dawley responses [F (1,104) = 3.243, p = 0.075]. Males had greater PPP than females [F (1, 214) =6.421, p = 0.01].

Percent Pre-Pulse to 122dB w/ Pre-Pulse. Figure 24 presents percent pre-pulse inhibition to the 122dB stimulus with pre-pulse on Experimental Days 2, 6, and 12, respectively. On **Day 2** nicotine's effects depended on stress [F (2, 214) = 3.313, p = 0.038] with 6 mg/kg/day nicotine decreasing PPP in non-stressed subjects but increasing PPP in stressed subjects. In addition, effects of nicotine depended on sex [F (2, 214) = 3.964, p = 0.02] with nicotine decreasing PPP for males but increasing PPP for females, especially among Long-Evans subjects [F (2, 107) = 5.450, p < 0.01]. Among female subjects nicotine had no effect on Sprague-Dawley PPP but increased female Long-Evans PPP [F (2, 105) = 3.374, p = 0.038]. For Long-Evans females this increase [F (2,53) = 3.697, p = 0.03] was the result of the 12 mg/kg/day nicotine group responses. Sprague-Dawley subjects exhibited more percent pre-pulse than Long-Evans subjects [F (1, 214) = 9.444, p < 0.01], especially among females [F (1, 105) = 8.575, p < 0.01]. Males exhibited more PPP than females [F (1, 214) = 6.644, p = 0.01], especially among Long-Evans subjects [F (1, 107) = 6.153, p < 0.05].

On **Day 6** Sprague-Dawley subjects exhibited greater PPP than Long-Evans subjects [F (1, 214) = 32.744, p < 0.01] whether subjects were male [F (1, 106) = 8.380, p < 0.05] or female [F (1, 105) = 25.793, p < 0.01]. Males exhibited greater PPP than

females [$\underline{F}$ (1, 214) = 4.768, p = 0.03], especially among Long-Evans subjects [$\underline{F}$ (1, 107) = 4.150, $p < 0.05$].

On **Day 12** Sprague-Dawley subjects exhibited greater PPP than Long-Evans subjects [$\underline{F}$ (1, 214) = 16.103, $p < 0.01$], especially among females [$\underline{F}$ (1, 105) = 17.872, $p < 0.01$]. Male and female Sprague-Dawley subjects exhibited similar PPP but male Long-Evans subjects exhibited greater PPP than female Long-Evans subjects [$\underline{F}$ (1, 214) = 4.542, p = 0.034]. Greater male PPP also was evident among Long-Evans subjects [$\underline{F}$ (1, 107) = 3.832, p = 0.05]

Confirmation of Hypotheses

Hypothesis 1 that nicotine administration would decrease body weight in a dose-response fashion for both strains of rats, with 12 mg/kg/day nicotine decreasing body weight more than 6 mg/kg/day nicotine was **confirmed**. These effects were similar in Sprague-Dawley males and females at both nicotine dosages. In Long-Evans subjects, in contrast to Experiment 1, nicotine's effects on body weight were greater in females (6 mg/kg/day = 9.26% reduction; 12 mg/kg/day = 12.0% reduction) than in males (6 mg/kg/day = 2.65% reduction; 12 mg/kg/day = 7.10% reduction) at both dosages.

Hypothesis 2 that Sprague-Dawley subjects would exhibit greater startle amplitudes and greater amounts of pre-pulse inhibition than Long-Evans subjects was **confirmed**. This pattern was observed in female as well as male subjects, extending the findings of Acri et al. (1995) to females.

Hypothesis 3 that nicotine administration would increase startle amplitude and amount of pre-pulse inhibition in Sprague-Dawley subjects but decrease startle amplitude and amount of PPI in Long-Evans subjects was **confirmed**. Significant startle amplitude Drug X Strain interactions and main effects for Drug in opposite directions for each strain were present on Days 2, 6 and 12. Similarly, significant pre-pulse amount Drug X Strain interactions and main effects for Drug in opposite directions for each strain were present on Days 2, 6, and 12.

Hypothesis 4 that restraint stress would increase startle and pre-pulse amounts in Sprague-Dawley males and Long-Evans females but decrease startle and PPI in Sprague-Dawley females and Long-Evans males was **confirmed** for Sprague-Dawley males and

females and **partially confirmed** for Long-Evans males and females. Specifically, effects of stress depended on sex as well as strain of subjects, and these responses sometimes also depended on drug treatment condition, day of experiment, and stimulus intensity.

For Sprague-Dawley males, stress increased startle and pre-pulse inhibition amounts regardless of day of experiment and stimulus intensity. Stress generally decreased startle amplitude and PPI amounts for Sprague-Dawley females. For Long-Evans males stress generally decreased responses but these effects sometimes depended on drug condition and reversed by Day 12. For Long-Evans females stress increased responses to the 98dB and 112dB stimuli but decreased responses to the 122dB stimulus.

Hypothesis 5 that restraint stress would combine with 12 mg/kg/day nicotine administration such that these subjects would have startle amplitudes and pre-pulse amounts indistinguishable from saline non-stressed subjects was **mostly confirmed** but confirmation depended on the strain and sex of the subject as well as on the stimulus to which responses were measured. Specifically, the hypothesis was **confirmed** for Sprague-Dawley males, **confirmed** for Long-Evans males except to the 122dB stimulus, and **confirmed** for Long-Evans females. The hypothesis was **not confirmed** for Sprague-Dawley females. Although responses of the two groups generally were indistinguishable to the 98dB stimulus, the stressed, nicotine-treated subjects exhibited greater responses than the non-stressed, saline-treated group to the other stimuli.

Discussion of Experiment 2

Experiment 2 replicated and extended the findings of Experiment 1. Specifically, Experiment 2 results indicated that there are robust strain differences in ASR and PPI responses to nicotine administration such that nicotine administration increased startle and pre-pulse inhibition in Sprague-Dawley subjects but decreased these responses in Long-Evans subjects. These effects occurred regardless of sex of subject.

The effects of stress on ASR and PPI responses depended on sex and strain of subject. Stress generally increased responses of male Sprague-Dawley subjects, decreased responses of female Sprague-Dawley subjects, interacted with drug dose for Long-Evans male subjects, and increased responses of female Long-Evans subjects except to the 122dB stimulus.

The effects of nicotine and stress together also depended on the strain and sex of subjects, with nicotine + stress together producing responses similar to saline non-stressed controls for Sprague-Dawley males and Long-Evans females, and for Long-Evans males except to the 122dB stimulus. For Sprague-Dawley females nicotine + stress generally produced responses greater than saline non-stressed control responses.

The effects of nicotine administration on body weight of males and females of both strains were consistent with published reports in that nicotine decreased body weight in a dose-response fashion. In addition, the effects of stress on body weight were striking. Sprague-Dawley and Long-Evans males were similarly affected by stress with body weight reductions of 6.0% and 5.0% respectively when mean body weights of saline non-stress subjects and saline stress subjects are compared. Sprague-Dawley females were least

affected by immobilization, with body weight decrements of only 1.78% between these two groups. Long-Evans females were significantly affected by stress, with body weight reductions of 9.0% in the saline stress group. When nicotine and stress were combined, males of each strain and Long-Evans females exhibited similar body weight reductions, but Sprague-Dawley females exhibited the smallest weight reductions. In fact, in the 12 mg/kg/day nicotine group the effects of stress attenuated the effects of nicotine, resulting in this group weighing more than the 12 mg/kg/day nicotine group without stress.

Overall, the results of Experiment 2 indicate that use of these two strains and both sexes may be a useful model for investigating individual differences relevant to human smoking behavior and to the effects of stress in smokers as well as in nonsmokers. In addition, the results suggest that the variables of genotype and gender should be considered in the use of nicotine as a therapeutic agent and in the development of nicotine analogs.

GENERAL DISCUSSION

Cigarette-smoking is a wide-spread, health-impairing behavior in the United States and throughout the world. Unexplained individual differences in smoking behavior exist, including differences in reported and empirically demonstrated effects of nicotine. These effects include changes in body weight, attenuation of stress, and changes in attention and cognitive performance. Better understanding of these individual differences in response to nicotine may help reveal why some people never smoke, others smoke intermittently, and still others spend a lifetime smoking and struggling to quit. In addition, explication of individual differences in smoking behavior may be relevant to possible use of nicotine or its analogs as therapeutic agents.

The extent to which individual differences in smoking behavior are biologically-based is not clear. Smoking prevalence data indicate that the proportion of smokers and amount of smoking varies within ethnic groups and between sexes within ethnicities. These prevalence differences could be explained by psychological or environmental factors. The differences also may, in part, result from genotypic differences in effects of nicotine experienced. Genotype, including the individual's gender, is a biologically-based individual difference that is relevant to drug effects. In humans genetic factors are major determinants of normal differences in drug responses among ethnicities and between genders (Matthews, 1995). In animals the strain and sex of subjects also can alter responses to drugs. In contrast to work with humans, animal models permit direct examination of the role of central processes.

Individual differences exist in nicotine's effects on attention and may depend on

genotype and gender. That is, surveys indicate that some but not all smokers report that smoking enhances attention and cognitive performance (Russell et al., 1974; USDHHS, 1988; Heishman et al., 1994). With regard to the role of genotype and gender, these studies are silent. Usually male subjects of predominantly of one ethnicity were tested. When female subjects were included (e.g., Provost & Woodward, 1991), the number of subjects per cell was insufficient to reliably distinguish sex differences.

Nicotine's effects on attention, operationalized in the acoustic startle reflex (ASR) and pre-pulse inhibition of the ASR, have been studied in different strains of rats. These two behavioral measures are believed to reflect processes of reactivity to external stimuli and sensory-gating that underlie the broader cognitive process of attention. In previous experiments with one albino rat strain -- Sprague-Dawleys -- nicotine enhanced reactivity to external stimuli and sensory-gating (Acri et al., 1991; Acri, 1992, 1994; Acri et al., 1994; Popke et al., 1994; Acri et al., 1995). This enhancement has been interpreted as analogous to the attentional enhancement demonstrated empirically in certain human subjects and reported by some human smokers when they smoke cigarettes. One study using Long-Evans subjects — a non-albino strain — also reported nicotine-induced enhancement of PPI (Curzon et al., 1994). The methodology of this study, however, differed on key variables that profoundly affect startle and PPI behaviors such as time of testing during the circadian cycle. Whether strain differences in nicotine's effects existed, therefore, was not clear. The nicotine cessation literature revealed similar inconsistencies. Studies using Sprague-Dawley subjects have found no effects of cessation on startle and reduced pre-pulse inhibition (Acri et al., 1991; Acri, 1992). Studies using Long-Evans

subjects, however, have reported that cessation enhances startle (Helton et al., 1993; Rasmussen et al., 1996). Whether strain differences existed in effects of nicotine cessation on ASR and PPI, therefore, also was not clear.

The role of sex of subject in attentional processes also had not been adequately examined in a rat model. In humans, men and women have been reported to exhibit similar startle behaviors but different PPI responses. Specifically, men exhibited greater PPI than women (Swerdlow et al., 1993). In women variations across the menstrual cycle also have been found (Swerdlow et al., 1997). Only one study has examined sex differences in rats. Using Sprague-Dawley subjects, Swerdlow and colleagues (1993) reported that males and females did not differ in startle or PPI behaviors. Responses of male and female Long-Evans subjects had not been tested within the same study. Therefore, the existence of sex differences within and across strains was neither established nor ruled out.

Environmental conditions that result in stress also can alter the effects of drugs and may interact with genotype and/or sex. For example, in Wistar rats housing condition can be a stressor depending on the sex of the animal. Specifically, males are stressed by crowded housing but females are stressed by individual housing (Brown & Grunberg, 1995). Whether housing condition interacted with sex in rat strains other than the Wistar strain was not known. Further, the effects of nicotine administration and cessation in different housing conditions were not known.

The non-painful physical stressor of immobilization differs from housing manipulation in that it has been reported to produce similar stress responses in males and

females (Kant et al., 1983). In male Sprague-Dawley rats nicotine administration attenuated the behavioral effects of this stressor as measured by the ASR and PPI paradigm (Acri, 1992; 1994). The nicotine-induced attenuation of stress' behavioral effects has been interpreted as support for smokers' reports that cigarette-smoking is stress-relieving despite the fact that nicotine increases levels of peripheral biochemical stress hormones. The mechanism by which cigarette-smoking might alleviate stress, therefore, is not clear. In addition, the effects of this stressor combined with chronic nicotine administration in female Sprague-Dawley subjects, or in males and females of other strains, is not known.

In summary, epidemiological data indicate the men and women of different genotypes exhibit different patterns of smoking behavior. Nicotine's effects on attentional processes is one area where individual differences exist. Stress also is relevant in this discussion because smokers report smoking is stress-relieving and stress is associated with relapse to smoking cessation. The acoustic startle reflex and pre-pulse inhibition provide a behavioral paradigm that can index basic attentional processes in humans and in rats and is sensitive to effects of nicotine and of stress. The role of genotype can be explored in an animal model with the use of different rat strains.

Experiment 1

The goal of Experiment 1, therefore, was to clarify effects of nicotine administration and cessation on ASR and PPI in Long-Evans subjects. A second goal was to determine whether males and females of this non-albino strain would respond differently to nicotine. A third goal was to examine whether ASR and PPI responses would be

altered by environmental conditions, and whether these effects would interact with subjects' sex and/or with nicotine administration and cessation.

Strain Differences. The most striking finding of Experiment 1 was that nicotine administration decreased startle amplitude and impaired PPI in Long-Evans subjects. These findings contrasted with previously reported findings in Sprague-Dawley subjects indicating that nicotine enhanced startle and PPI (Acri et al., 1991; Acri, 1992, 1994; Grunberg et al., 1994; Popke et al., 1994; Acri et al., 1995). In addition, these findings contrasted with data from Long-Evans subjects obtained during the light portion of the circadian cycle (Curzon et al., 1994). These findings suggested, therefore, a strain difference in nicotine's attentional effects between Sprague-Dawley and Long-Evans subjects.

Sex Differences. Long-Evans males and females differed in their PPI responses to nicotine administration and to housing conditions in several ways. For females nicotine administration generally impaired PPI regardless of housing condition. In contrast, for males nicotine's effects always depended on housing condition, with nicotine enhancing PPI in crowded subjects but impairing PPI in individually-housed subjects. Nicotine's effects in male and female subjects also followed different time courses. Specifically, nicotine-induced startle reductions appeared robustly in females on Day 6 but did not appear in males until Day 11. Housing effects showed the opposite pattern. Drug X Housing Condition interactions were evident for males on Day 6, but were not evident in females until Day 11 and in Cessation. Taken together, these results are consistent with females having greater sensitivity to nicotine's effects than males. That is, females were

affected more quickly than males by nicotine administration, effects of housing condition on females were minimal compared to effects of nicotine, and effects of housing on females did not appear until Day 11. In contrast, males took longer to be affected by nicotine, drug effects always depended on housing, and housing effects appeared on Day 6, suggesting that for males the environmental manipulation was a more powerful determinant of responses.

Apart from effects of nicotine, males and females also responded differently to the two stimuli (112 and 122dB). Males responded in an increasing, linear fashion to acoustic stimuli, with maximal responses occurring to the loudest stimulus. Maximal responses for females, however, depended on environmental conditions as well as on stimulus intensity. These results contrast with other reports that Sprague-Dawley males and females do not differ in startle or PPI behaviors (Swerdlow et al., 1993). These sex differences in reactivity to external stimuli and in sensory-gating also suggest that male and female responses to environmental stimuli (e.g., noises of different intensities) may be qualitatively different.

Overall, Experiment 1 results replicated other investigators' findings that startle and pre-pulse inhibition are separately manipulable by drugs (Davis et al., 1975; Pohorecky et al., 1976; Davis, 1988; Swerdlow et al., 1986; Mansbach et al., 1988; Swerdlow et al., 1990; 1992). In addition, these results extend this literature by indicating that startle and PPI also are separately affected by housing conditions.

Experiment 2

Experiment 2 was undertaken because of the surprising principal finding from

Experiment 1, i.e., that nicotine administration impaired startle and PPI in Long-Evans subjects. The goal of Experiment 2 was to replicate and extend the findings of Experiment 1 by explicitly examining genotype as an independent variable. In Experiment 2, therefore, the effects of nicotine on ASR and PPI in Sprague-Dawley and Long-Evans male and female subjects were investigated. Because effects of nicotine cessation were minimal in Experiment 1, cessation effects were not examined in Experiment 2.

In addition, two other methodological changes were made. First, because housing condition had been conceptualized as a stressor in Experiment 1 but did not produce behavioral responses consistently identifiable as stress responses as predicted, the effects of a different environmental variable -- immobilization stress -- with and without nicotine were investigated in Experiment 2. This nonpainful physical stressor was used in order to examine effects of a manipulation that has reliably produced similar biochemical stress responses in males and females (Kant et al., 1983).

Second, Experiment 1 results were consistent with Long-Evans subjects having greater sensitivity to nicotine's effects on ASR and PPI than reported effects in Sprague-Dawley subjects, i.e., the Long-Evans dose-response curve would be shifted to the left of the Sprague-Dawley dose-response curve. Therefore, in order to determine the relative placement of Sprague-Dawley vs. Long-Evans dose-response curves, three doses of nicotine (0 mg/kg/day, 6 mg/kg/day, and 12 mg/kg/day) were used.

Strain Differences. Experiment 2 results replicated past work with Sprague-Dawley subjects and replicated and extended Experiment 1 results. Nicotine administration generally impaired startle and sensory-gating in Long-Evans subjects and

enhanced startle and sensory-gating in Sprague-Dawley subjects. In addition, for Sprague-Dawley subjects nicotine's startle and pre-pulse inhibition enhancing effects generally occurred in a dose-response pattern with the greatest effects evident at the 12 mg/kg/day nicotine dose. For Long-Evans subjects, however, the 6 mg/kg/day and 12 mg/kg/day nicotine dose generally decreased startle and PPI responses to similar extents. Effects of nicotine in Long-Evans subjects, therefore, did not follow a dose-response pattern.

One interpretation of these data is that the dose-response curve for Long-Evans subjects is so far to the left of the Sprague-Dawley dose-response curve that the 6 mg/kg/day dose produced maximal behavioral suppression. That is, in comparison with Sprague-Dawley subjects, Long-Evans subjects might be exquisitely sensitive to nicotine's effects on these behavioral measures. The 12 mg/kg/day dose, then, also would result in behavioral suppression but because the behavior is reduced to its lowest point by the 6 mg/kg/day dose -- a floor effect -- no additional suppression would be observed. If this interpretation is correct, then lower doses of nicotine -- e.g., 1 or 3 mg/kg/day -- might produce enhancement of startle and PPI. This hypothesis can be tested by examining lower nicotine doses in the Long-Evans strain and constructing a more detailed dose-response curve. It also is possible that nicotine's effects on ASR and PPI in Long-Evans subjects simply follow a differently shaped dose-response curve than in Sprague-Dawley subjects.

The two strains also exhibited different temporal responses to nicotine administration. Drug effects were present in both strains on Days 2 and 6 of the drug

administration period. By Day 12, however, drug effects remained in Sprague-Dawley subjects but were largely absent in Long-Evans subjects. These data suggest that Long-Evans subjects developed tolerance to nicotine's effects faster than did Sprague-Dawley subjects. Recent work in humans and animals suggests that vulnerability to nicotine dependence is related to high initial sensitivity to nicotine and rapid tolerance development (Pomerleau, 1995). If so, then it is possible that use of these two strains to study nicotine's effects constitutes an animal model of more vulnerable (Long-Evans) and less vulnerable (Sprague-Dawley) smokers. In any case, these genotypic differences make clear that strain of experimental subject may profoundly affect results because of different baseline responses as well as qualitatively different responses to drug manipulations.

Sex Differences. Within each strain, male and female subjects also exhibited different time courses of responses to nicotine administration. Regardless of strain, female behavior was affected more quickly (on Day 2) and across more measures by nicotine administration than was male behavior and disappeared more quickly (by Day 12). In contrast, effects on male behavior appeared later (on Day 6) and persisted longer (present on Day 12). These results replicated Experiment 1 findings for Long-Evans subjects and indicate that the greater sensitivity of females occurs regardless of subjects' strain.

Within both strains male and female subjects responded differently to each stimulus (98, 112, and 122dB) and to each stimulus with pre-pulse. In general, female Long-Evans subjects startled more to a given stimulus than male Long-Evans subjects but male Long-Evans subjects exhibited greater amounts of pre-pulse than female Long-Evans subjects. In contrast, Sprague-Dawley males and females startled similar amounts to each stimulus

but male Sprague-Dawley subjects exhibited greater pre-pulse amounts than female Sprague-Dawley subjects. These data suggest that startle responses do not depend on sex in the albino strain but are altered by sex in the non-albino strain. This result replicates Swerdlow and colleagues (1993) finding that Sprague-Dawley males and females startled to similar extents. The fact that males of each strain exhibited greater sensory-gating -- and possibly greater attentional processing -- than females of each strain contrasts with past reports in Sprague-Dawley subjects of no sex differences in PPI but does mirror human sex differences in PPI (Swerdlow et al., 1993). Further, this sex difference was exaggerated in the Long-Evans strain. That is, male Long-Evans subjects startled less than female Long-Evans but their pre-pulse amounts were greater. Taken together, these results suggest a qualitative difference in addition to a quantitative difference in responses to and gating of acoustic stimuli in males and females of different strains.

Stress. It is striking that the nonpainful physical stressor of immobilization, reported to result in similar biochemical stress responses in males and females, resulted in very different behavioral responses across sex and genotype. Specifically, males and females of different genotypes were differentially sensitive to this stressor. Stress altered Long-Evans male and female responses at the same time points (Days 2 and 12) but affected male Sprague-Dawley subjects throughout the experiment (Days 2, 6, and 12) and female Sprague-Dawleys only at the end of the experiment (Day 12). Male Sprague-Dawleys, therefore, exhibited the most consistent behavioral alterations in response to this type of stress and may be the most sensitive genotype-sex group.

Genotype and sex also altered the directional effects of restraint stress on ASR and

PPI. Stress generally decreased responses of Long-Evans males but these effects sometimes depended on drug condition and reversed by Day 12. For Long-Evans females, however, stress generally increased responses regardless of drug dose except to the 122dB stimulus. For Sprague-Dawley subjects stress generally increased male responses, replicating Acri (1992, 1994), and decreased female responses, replicating Popke et al., (1994). The genotype and sex of subject, therefore, determined whether stress improved or impaired reactivity and sensory-gating.

The effects of stress and concurrent nicotine administration also depended on the genotype and sex of subject. When 12 mg/kg/day subjects were subjected to stress, responses of Sprague-Dawley males, Long-Evans males, and Long-Evans females were indistinguishable from saline, non-stress control responses. These findings are consistent with smokers' reports that smoking alleviates stress despite the fact that nicotine administration elevates physiological and biochemical stress indices. For Sprague-Dawley females, however, nicotine administration and stress together resulted in startle and PPI responses greater than responses of saline-treated, non-stress subjects. Sprague-Dawley females, therefore, may exhibit responses similar to smokers who do not report stress attenuation from cigarette smoking.

Possible Mechanisms and Future Directions

Different behavioral responses by rats of different genotypes, of each sex, and exposed to different environmental conditions may mirror human individual differences in reported effects of smoking and of stress. To the extent that this is so, the conclusion that genotype, broadly construed to include subjects' sex, can alter responses to nicotine and

to stress is supported. This conclusion is useful, but describes rather than explains a phenomenon.

Knowledge of mechanisms -- the neurobiological underpinnings of differences in behavior -- is important in order to better understand individual differences in susceptibility to nicotine addiction. In addition, this knowledge has potential clinical relevance in two ways. First, knowledge of mechanisms may enhance the tailoring of smoking cessation therapies to individual needs for maximal success. Second, individual differences in effects of nicotine that depend on sex and genotype may be important in the use of nicotine and the development of nicotine analogs for use in disorders characterized by cognitive impairments such as Alzheimer's disease. Altered responses based on genotype may depend on peripheral or central mechanisms, or on both mechanisms. That is, Long-Evans and Sprague-Dawley male and female subjects may respond differently to nicotine administration as a result of differences in nicotine metabolism (peripheral mechanism) or different nicotine actions in the central nervous system (central mechanism) or an interaction between these two mechanisms.

Studies examining peripheral metabolism in rodents as mechanisms for behavioral differences in response to chronic nicotine administration, however, have not found metabolic differences in rats (Takeuchi, Kurogochi, & Yamaoka, 1954) or in mice (Hatchell & Collins, 1977; Marks, Burch, & Collins, 1983). Therefore, different behaviors in response to nicotine administration and different time courses of tolerance development must be the result of changes in central tissue sensitivity rather than changes in peripheral nicotine metabolism.

Strain differences in responses, then, may occur as a result of differences in number, affinity, or distribution of central nicotinic cholinergic receptors, differences in up- or down-regulation processes, or as a result of some combination of these factors. In the rodent brain at least two classes of nicotinic cholinergic receptors (nAChRs) exist. The ^{3}H-nicotine probe labels high-affinity, $\alpha4\beta2$-type neuronal receptors (Romano and Goldstein, 1980; Marks & Collins, 1982; Alkondon et al., 1994; Barrantes, Rogers, Lindstrom, & Wonnacott, 1995; Piccioto et al., 1995) . A second group of nAChRs -- the $\alpha7$-type receptor — is labeled by the snake toxin α-^{125}I-bungarotoxin (Morley, Kemp, & Salvaterra, 1979; Morley, Lorden, Brown, & Kemp, 1977) and has recently been isolated from the human cortex (Pereira et al., 1997). Marks et al. (1989) examined receptor number (B_{max}) and binding affinity (K_D) in eight brain areas of 19 inbred strains of mice known to exhibit behavioral differences in response to nicotine. Binding affinity did not differ among strains for either receptor type but significant differences across strain were found in receptor numbers, especially in midbrain, hindbrain, hippocampus, hypothalamus, and colliculus. Mouse strains with the greatest behavioral sensitivity to nicotine also had the greatest numbers of nicotinic cholinergic receptors. Comparable studies have not been done across rat strains but it is possible that similar processes underlie behavioral differences in response to nicotine administration.

Importantly, both receptor subtypes have been implicated in nicotine's effects on cognitive processes. Specifically, in mice the $\alpha4\beta2$ nAChR has been demonstrated to mediate nicotine's effects on startle behavior (Miner et al., 1986; Marks et al., 1989; Miner & Collins, 1989; Grun, Pauly, Bullock, & Collins, 1995). In addition, development

of a knock-out mouse strain in which the α4β2 nAChR is not expressed has indicated that nicotine administration in these animals fails to improve passive avoidance performance, a memory task (Piccioto et al., 1995). Although the molecular mechanism for nicotine's effects on sensory-gating (i.e., PPI) is not known, Albuquerque and colleagues (1997) have determined that the α7 nAChR presynaptically modulates release of glutamate and GABA, two neurotransmitters implicated in PPI regulation. It is possible, therefore, that changes in the α7 nAChR system are the mechanism for nicotine's effects on PPI. In addition, the α7 nAChR is sensitive to the stress hormone corticosterone, whereas the α4β2 nAChR is not (Pauly, Grun, & Collins, 1990; Grun, Pauly, & Collins, 1992; Grun et al., 1995). This sub-system, then, also may mediate the effects of stress and the effects of nicotine and stress together on PPI.

Future studies, therefore, should examine distribution, affinity, number, and functionality of the α4β2 nAChR and α7 nAChR receptor systems in male and female Sprague-Dawley and Long-Evans subjects in response to nicotine administration, stress, and nicotine + stress manipulations. Delineation of possible receptor level differences may reveal the mechanisms of the observed strain and sex differences in ASR and PPI responses and also may be relevant to human clinical issues.

Future studies also should examine within-strain within-sex variability. Some effects of nicotine may depend on the subject's baseline. For example, Rosecrans (1971, 1972) reported that nicotine's within-strain, within-sex effects on locomotion and central serotonin turnover depended on baseline activity levels. With regard to ASR and PPI, the work reported here and elsewhere has found individual differences in ASR and PPI

responses at baseline. These differences also may be relevant to nicotine's effects. Specifically, Acri (1994) reported that effects of nicotine and effects of stress to enhance Sprague-Dawley male responses were greater in subjects who exhibited initially greater startle and PPI. In addition, studies with human females indicate that attentional processes (Broverman et al., 1981; Swerdlow et al., 1997) may vary across the menstrual cycle and drug effects on these processes also may vary (Erickson et al., 1985; Arnold et al., 1987). Therefore, studies with female rats and humans should be conducted to examine nicotine's attentional effects at different stages of the estrous and menstrual cycles respectively. Finally, future studies should examine ASR and PPI responses within and across human genotypes, in smokers and nonsmokers, with and without exposure to stress.

It is striking that people tinker with their states of attention and consciousness routinely. If one is tired, a cup of coffee energizes. If one is depressed, a chocolate bar may lighten mood. If one is a smoker, and stressed, a cigarette may smooth and focus cognitive processes. In each case an alteration in subjective, psychological experience of the environment is sought and effected while the objective physical environment remains unchanged. The fact that these effects occur in some but not all smokers points toward the larger issue of the role of biologically-based individual differences (e.g., gender, genotype) in subjective experience. The extent to which differences in behavior, thought, and emotion depend on the idiosyncratic neuroanatomy and neurochemistry of the individual brain is unclear. Quantification of the biological portion of these differences, however, may be the key to understanding the psychological experience of another as well

as the extent to which consciousness itself can be known and dissected. These potential insights have implications for addictive behaviors, for clinical conditions, and for everyday understanding of motivations and actions.

TABLES

Table 1: Design of Experiment 1

Sex (2)	**X**	**Housing (2)**	**X**	**Drug (2)**	**X**	**Phase (2)**
Male (n = 96)		Individual (n = 48)		0 mg/kg/day (n = 24) 12 mg nic/kg/day (n = 24)		During (n = 12) Cessation (n = 12) During (n = 12) Cessation (n = 12)
		Crowded (n = 48)		0 mg/kg/day (n = 24) 12 mg nic/kg/day (n = 24)		During (n = 12) Cessation (n = 12) During (n = 12) Cessation (n = 12)
Female (n = 96)		Individual (n = 48)		0 mg/kg/day (n = 24) 12 mg nic/kg/day (n = 24)		During (n = 12) Cessation (n = 12) During (n = 12) Cessation (n = 12)
		Crowded (n = 48)		0 mg/kg/day (n = 24) 12 mg nic/kg/day (n = 24)		During (n = 12) Cessation (n = 12) During (n = 12) Cessation (n = 12)

N = 192

Table 2: Timeline of Experiment 1

BASELINE PHASE	— Days 1 - 3: Gentling
	— Days 4 - 6: ASR-PPI chamber acclimation
	— Days 7 - 10: ASR-PPI stimuli acclimation
	— Days 11-14: ASR-PPI Baseline measure
DRUG ADMINISTRATION PHASE	— Drug Administration Day 1: IMPLANT
	— Drug Administration Day 2: HOUSING MANIPULATION began
	— Drug Administration Day 6: ASR-PPI Measurement for all subjects (N = 192)
	— Drug Administration Day 12: ASR-PPI Measurement for During Phase subjects (n = 96)
	— Drug Administration Day 13: During Phase subjects sacrificed
	— Drug Administration Day 15: EXPLANT of Cessation Phase subjects (n = 96)
CESSATION PHASE	— Cessation Day 3: ASR-PPI measurement for Cessation Phase subjects
	— Cessation Day 5: Cessation Phase subjects sacrificed

Table 3: Design of Experiment 2

Strain (2)	**X**	**Sex (2)**	**X**	**Stress (2)**	**X**	**Drug (3)**
Long-Evans (n = 120)		Male (n = 60)		No Stress (n = 30)		0 mg/kg/day (n = 10) 6 mg nic/kg/day (n = 10) 12 mg nic/kg/day (n = 10)
				Stress (n = 30)		0 mg/kg/day (n = 10) 6 mg nic/kg/day (n = 10) 12 mg nic/kg/day (n = 10)
		Female (n = 60)		No Stress (n = 30)		0 mg/kg/day (n = 10) 6 mg nic/kg/day (n = 10) 12 mg nic/kg/day (n = 10)
				Stress (n = 30)		0 mg/kg/day (n = 10) 6 mg nic/kg/day (n = 10) 12 mg nic/kg/day (n = 10)
Sprague-Dawley (n = 120)		Male (n = 60)		No Stress (n = 30)		0 mg/kg/day (n = 10) 6 mg nic/kg/day (n = 10) 12 mg nic/kg/day (n = 10)
				Stress (n = 30)		0 mg/kg/day (n = 10) 6 mg nic/kg/day (n = 10) 12 mg nic/kg/day (n = 10)
		Female (n = 60)		No Stress (n = 30)		0 mg/kg/day (n = 10) 6 mg nic/kg/day (n = 10) 12 mg nic/kg/day (n = 10)
				Stress (n = 30)		0 mg/kg/day (n = 10) 6 mg nic/kg/day (n = 10) 12 mg nic/kg/day (n = 10)

N = 240

Table 4: Timeline of Experiment 1

BASELINE PHASE	— Days 1 - 3: Gentling
	— Days 4 - 6: ASR-PPI chamber acclimation
	— Days 7 - 10: ASR-PPI stimuli acclimation
	— Days 11-14: ASR-PPI Baseline measure
DRUG ADMINISTRATION PHASE	— Drug Administration Day 1: IMPLANT
	— Drug Administration Day 2: STRESS MANIPULATION begins (i.e., subjects in stress cells undergo 20 min/day IM stress beginning today; ASR-PPI Measurement for all subjects (N = 240)
	— Drug Administration Day 6: ASR-PPI Measurement for all subjects (N = 240)
	— Drug Administration Day 12: ASR-PPI Measurement for all subjects (N = 240)
	— Drug Administration Day 15: All subjects sacrificed

FIGURES

Figure 1a: During Phase Males - Body Weight

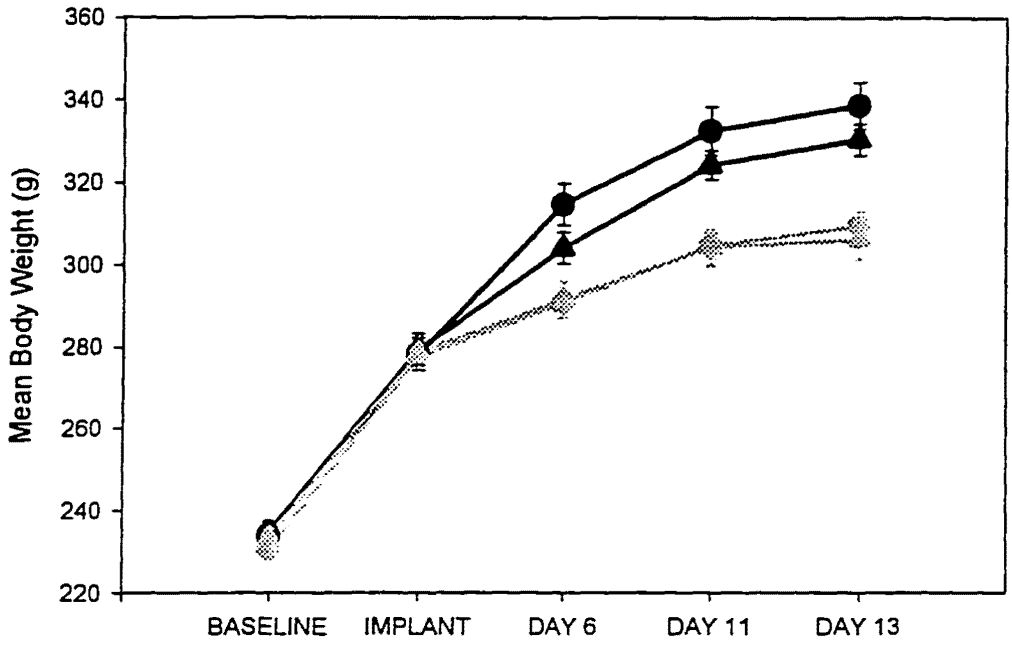


Figure 1b: During Phase Females - Body Weight

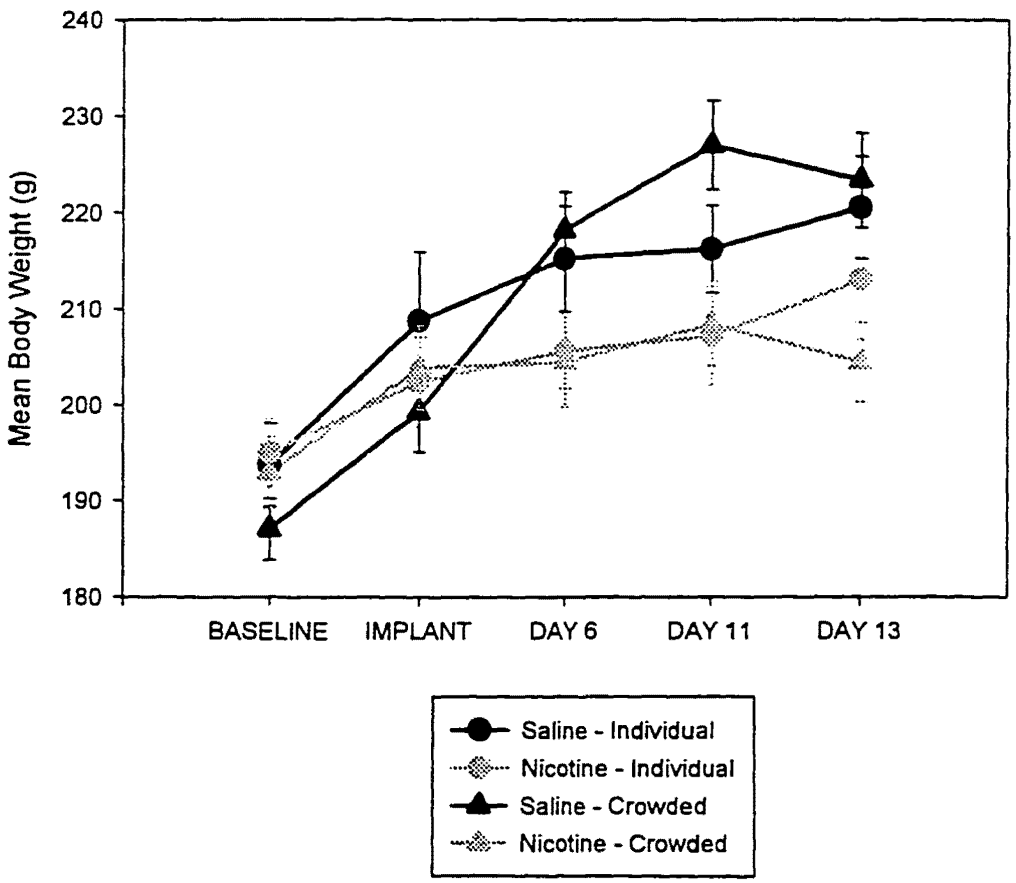


Figure 2a: Cessation Phase Males - Body Weight

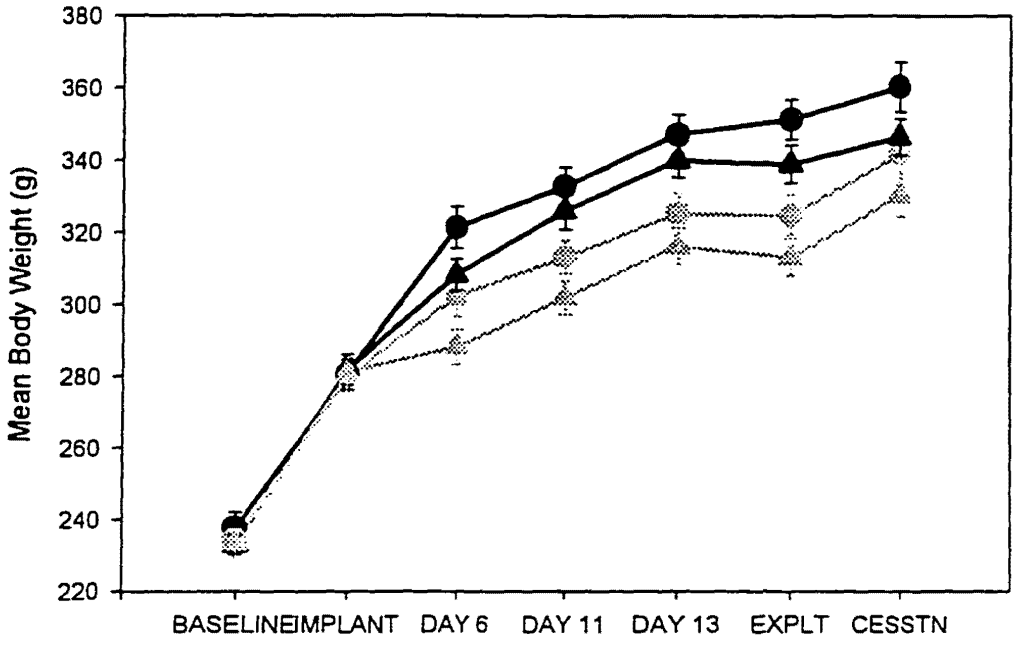


Figure 2b: Cessation Phase Females - Body Weight

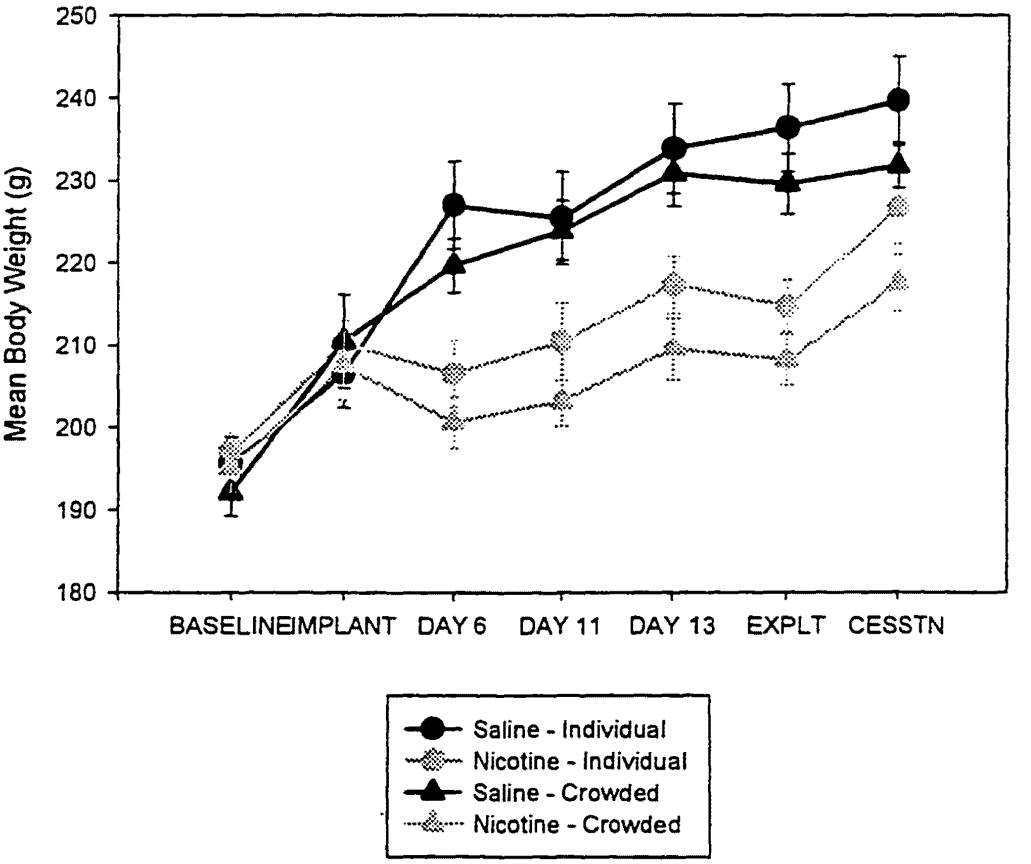


Figure 3a: Day 6 Startle Amplitude to 112dB Stimulus

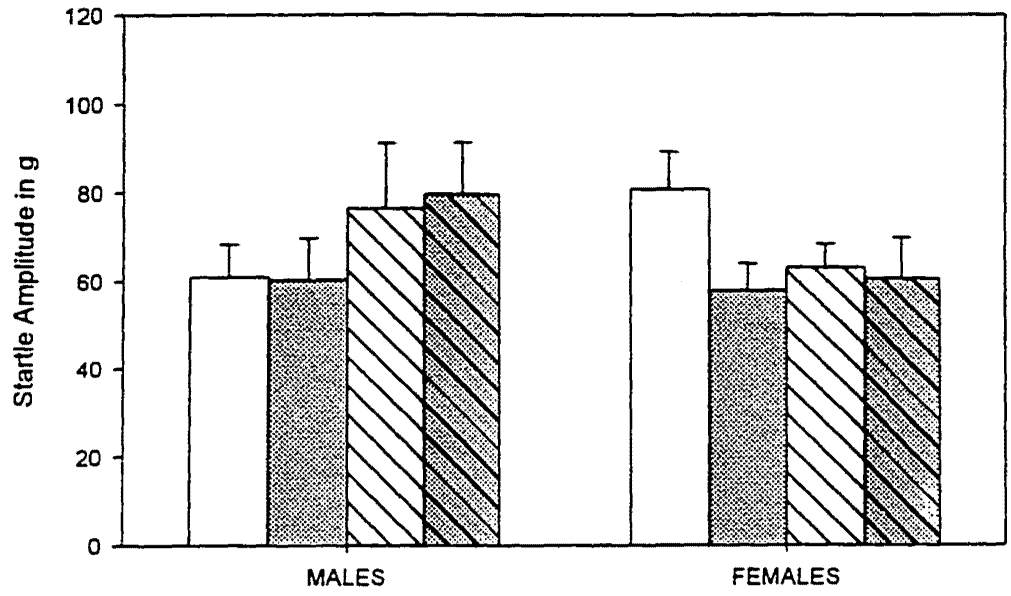


Figure 3b: Day 11 Startle Amplitude to 112dB Stimulus

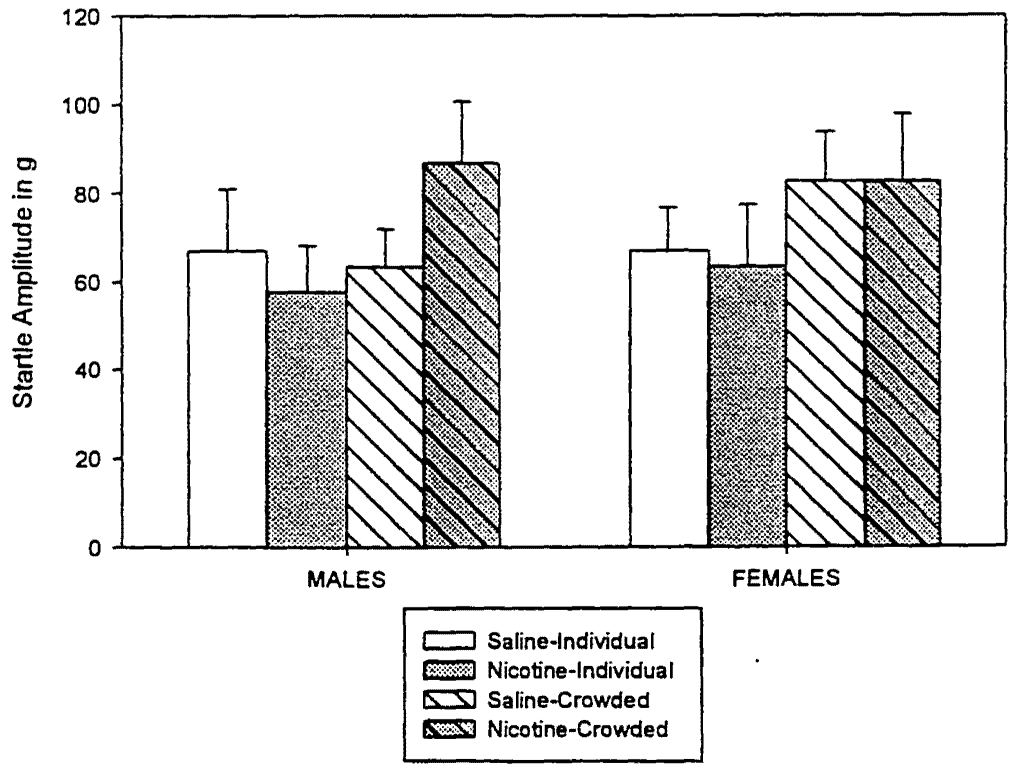


Figure 4a: Day 6 Startle Amplitude to 122dB Stimulus

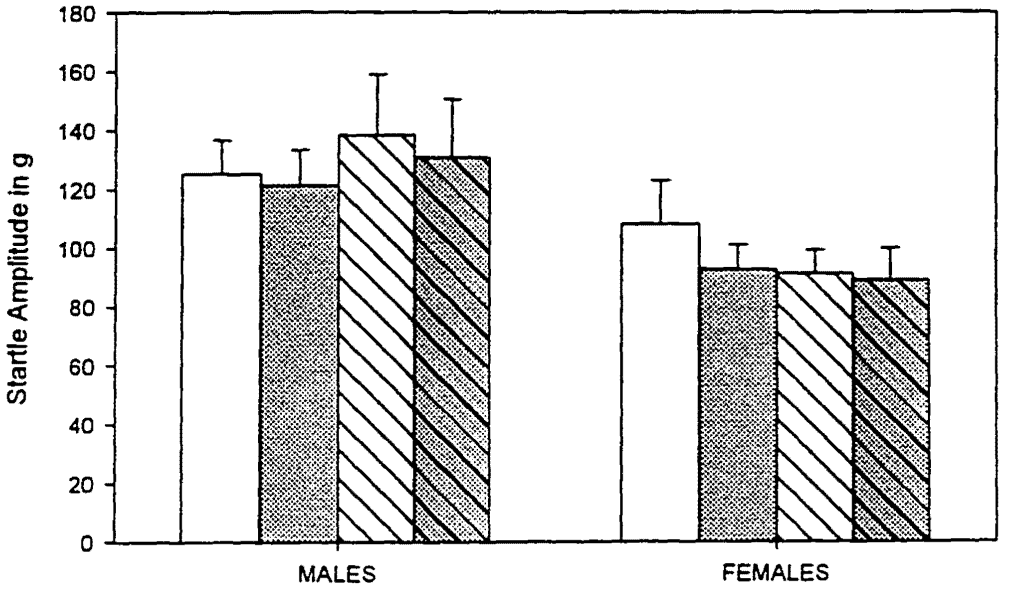


Figure 4b: Day 11 Startle Amplitude to 122dB Stimulus

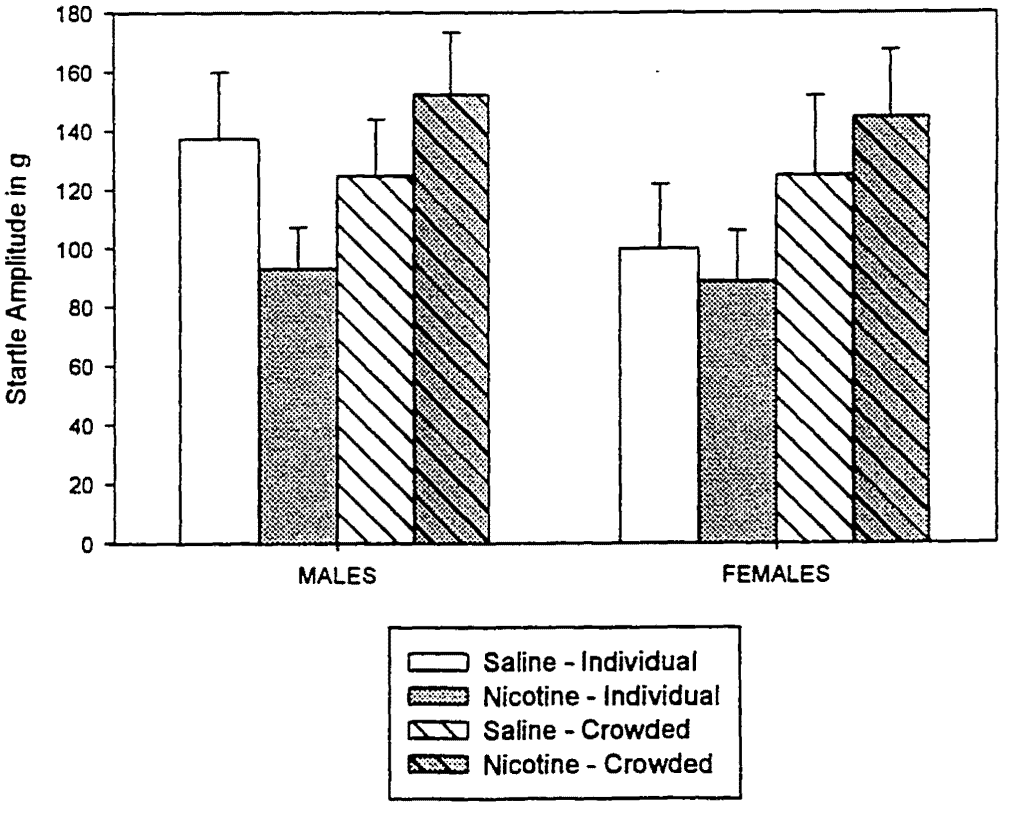


Figure 5a: Day 6 Pre-Pulse Inhibition Amount to 112dB

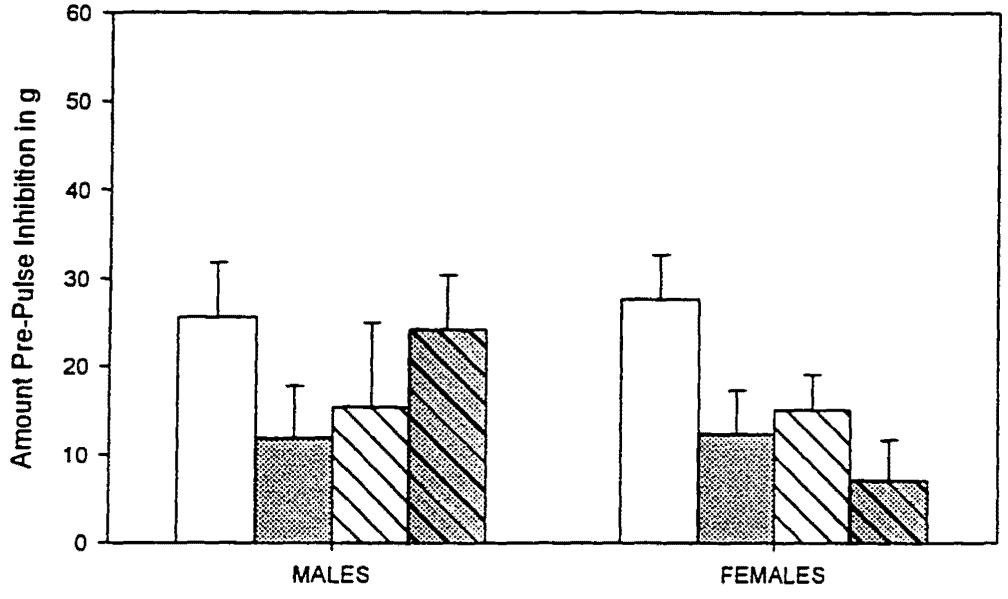


Figure 5b: Day 11 Pre-Pulse Inhibition Amount to 112dB

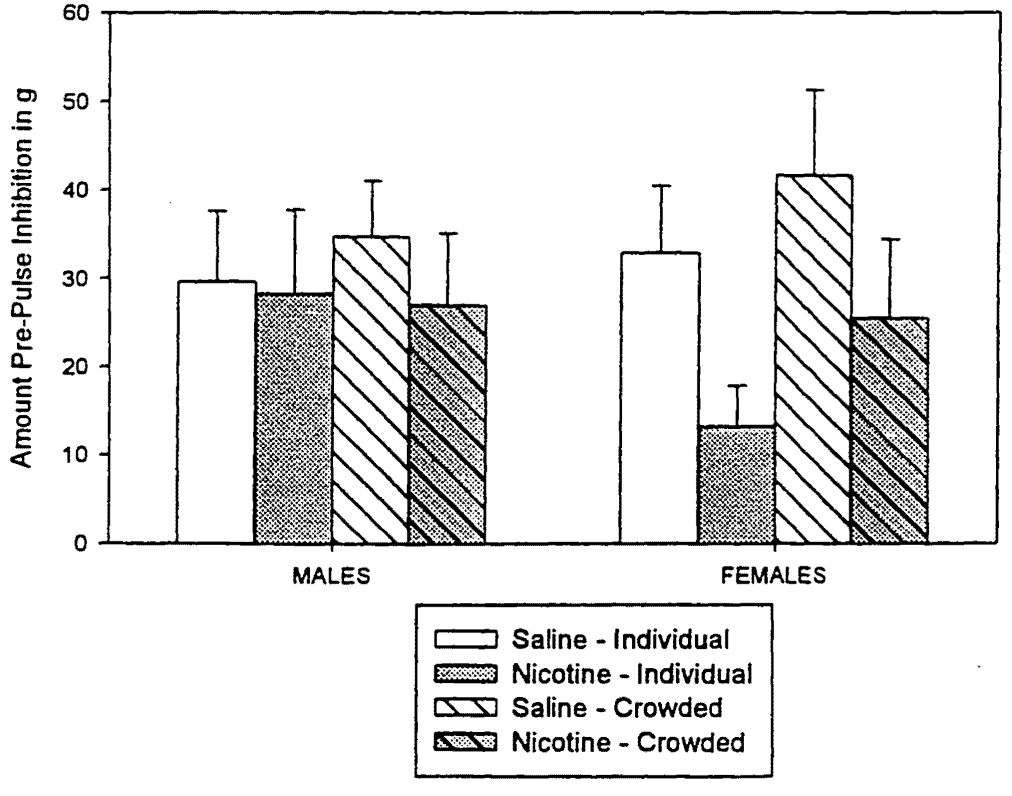


Figure 6a: Day 6 Pre-Pulse Inhibition Amount to 122dB

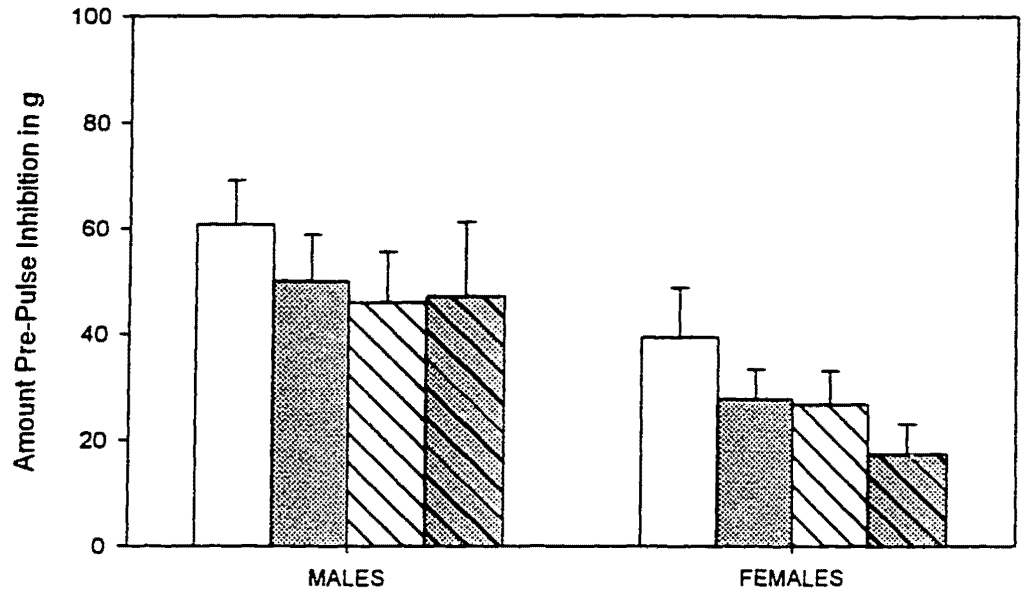


Figure 6b: Day 11 Pre-Pulse Inhibition Amount to 122dB

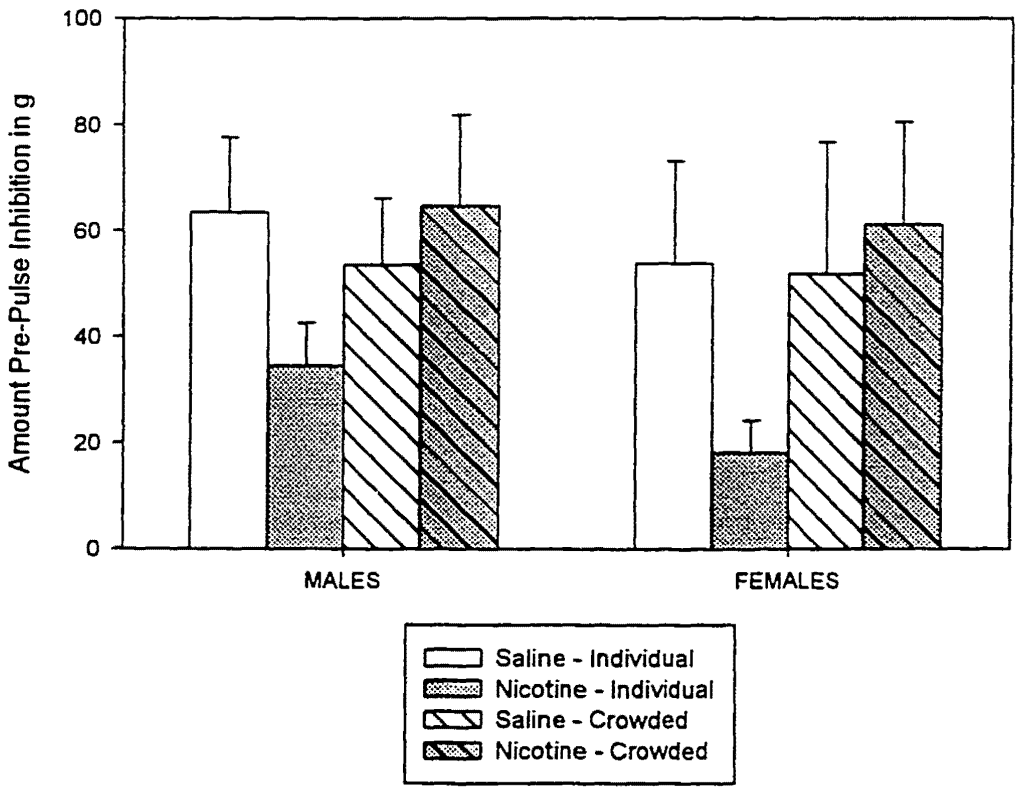


Figure 7a: Day 6 Percent Pre-Pulse Inhibition to 112dB

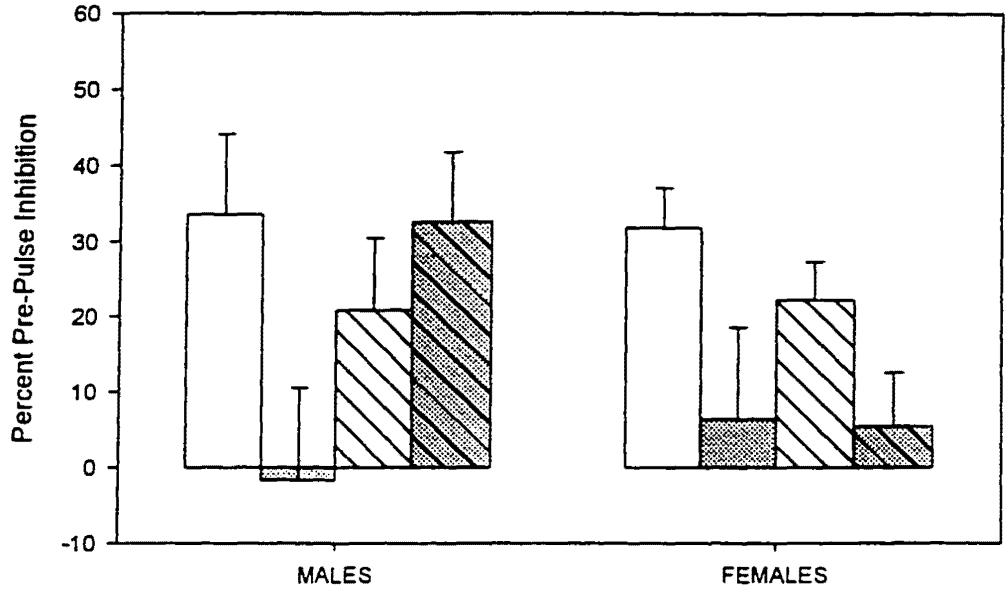


Figure 7b: Day 11 Percent Pre-Pulse Inhibition to 112dB

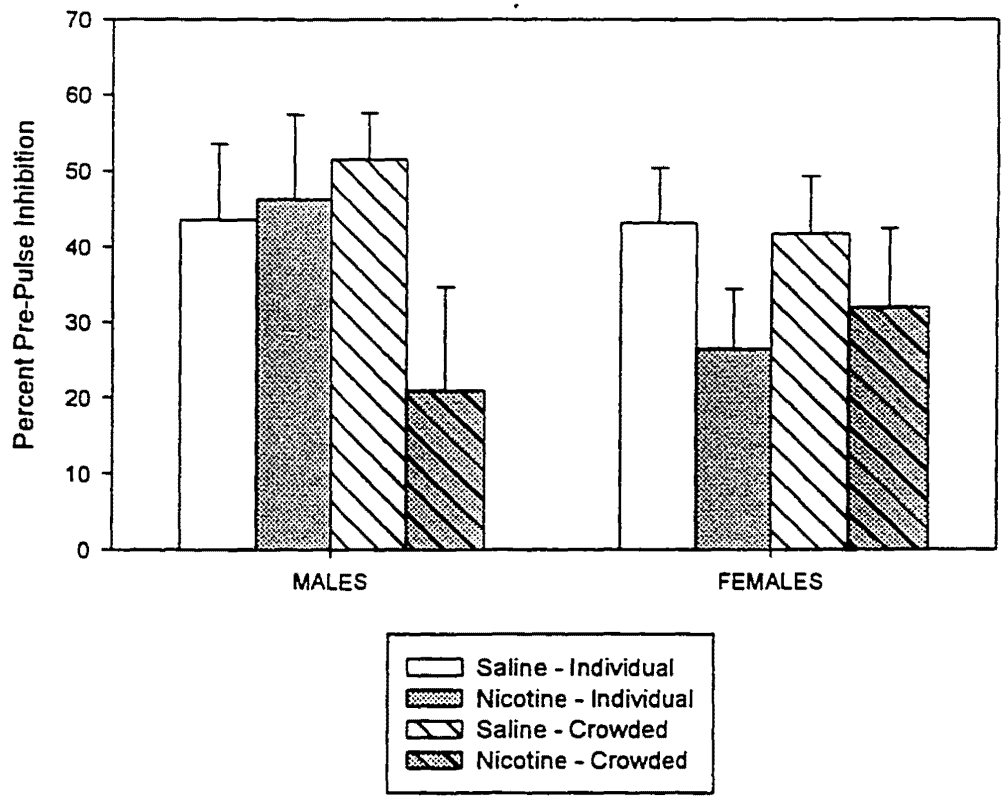


Figure 8a: Day 6 Percent Pre-Pulse Inhibition to 122dB

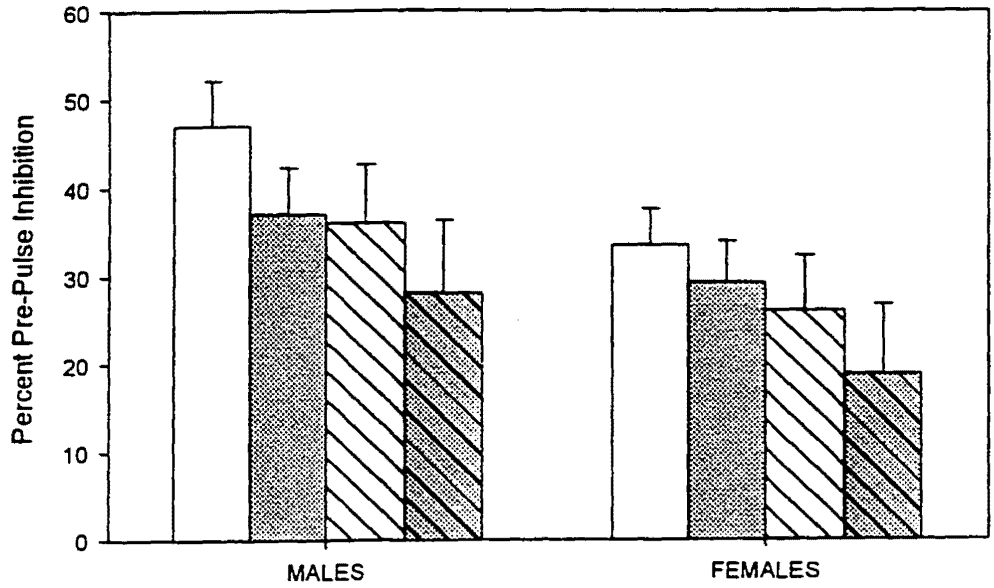


Figure 8b: Day 11 Percent Pre-Pulse Inhibition to 122dB

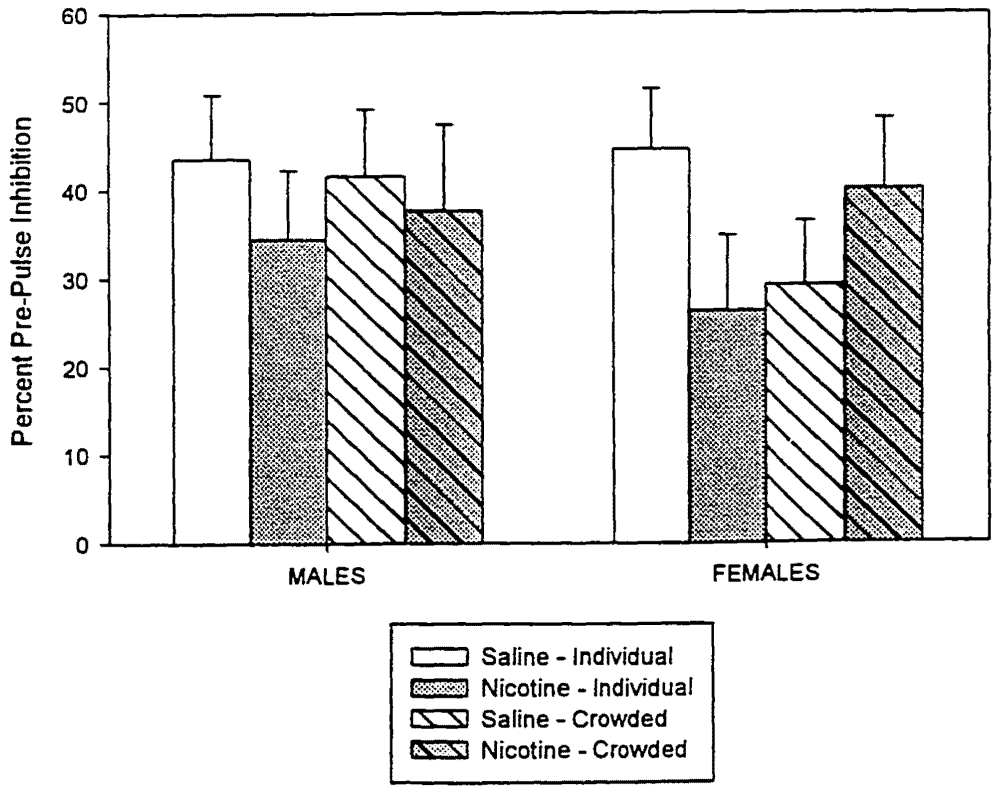


Figure 9: Cessation Day 3 Startle Amplitude to 112dB Stimulus

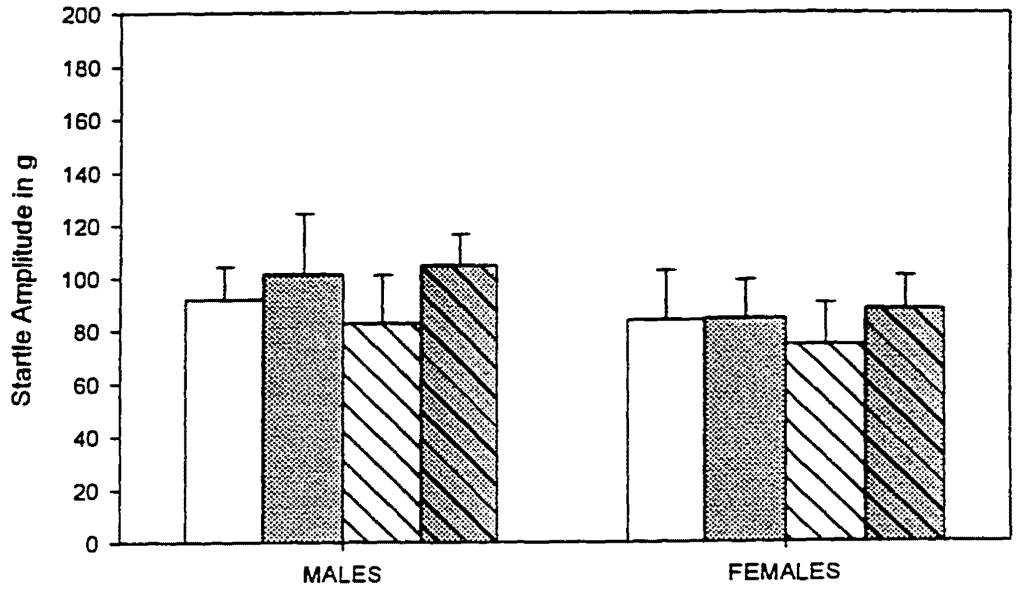


Figure 10: Cessation Day 3 Startle Amplitude to 122dB Stimulus

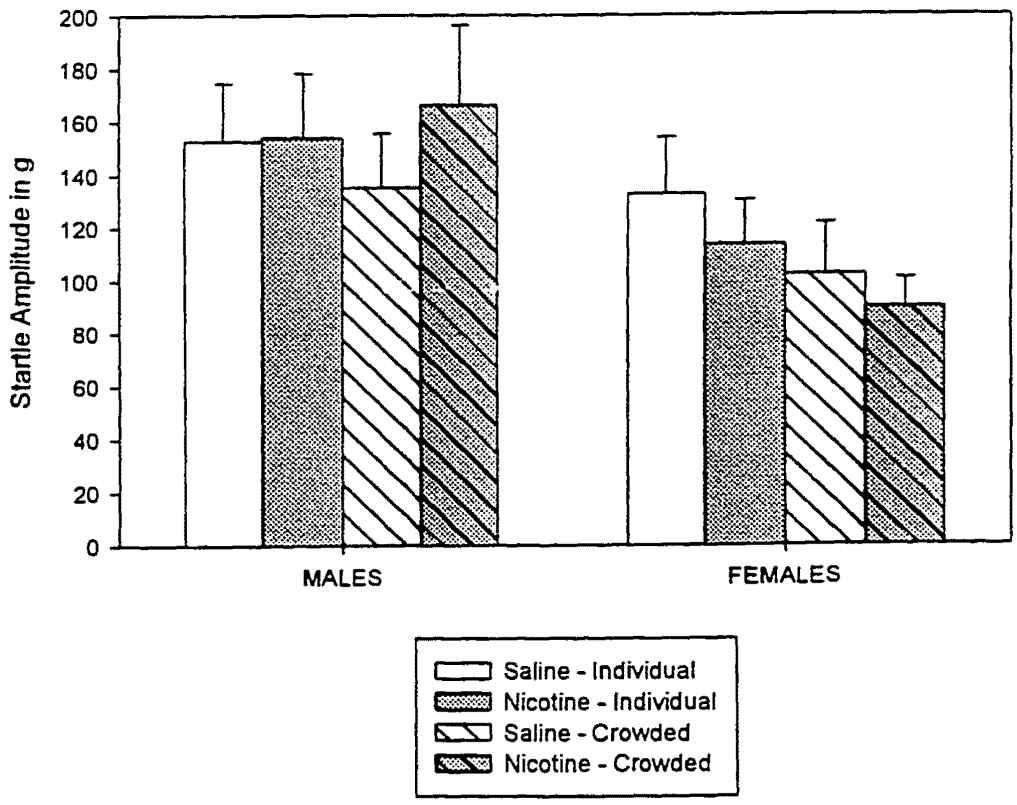


Figure 11: Cessation Day 3 Pre-Pulse Inhibition Amount to 112dB

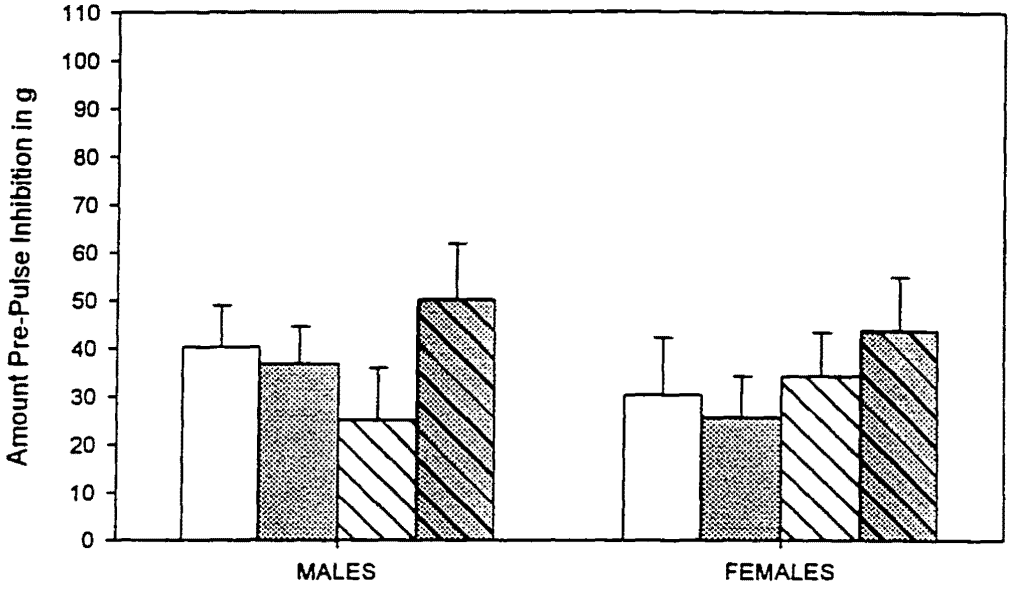


Figure 12: Cessation Day 3 Pre-Pulse Inhibition Amount to 122dB

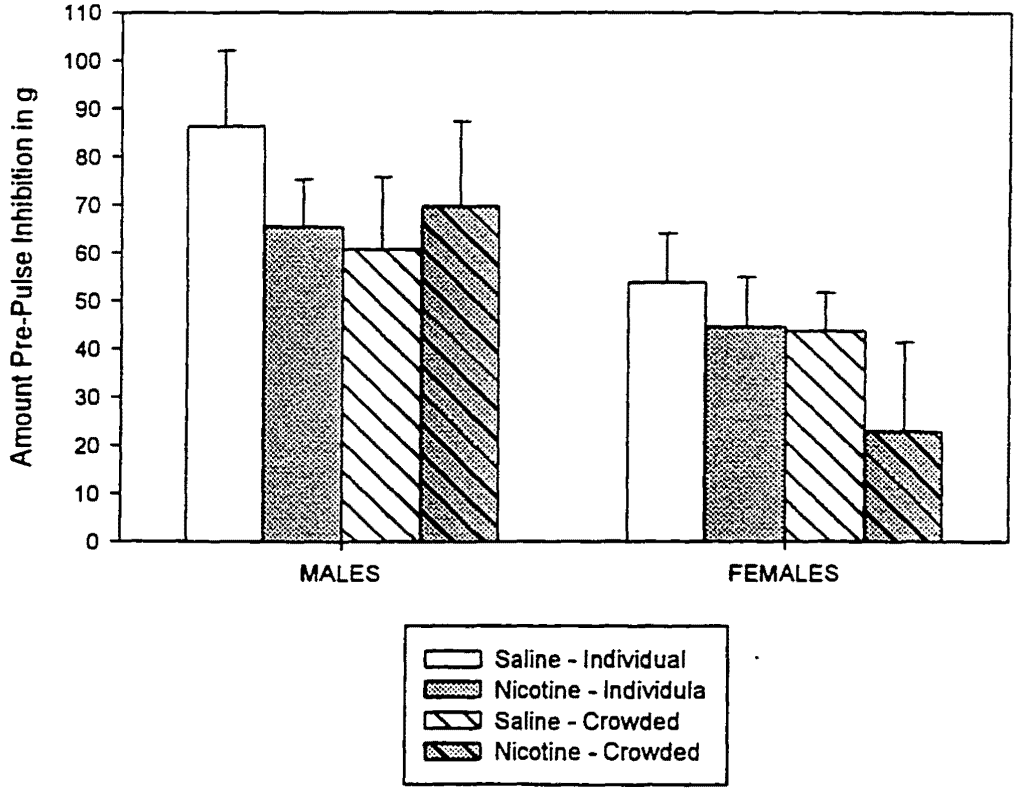


Figure 13: Cessation Day 3 Percent Pre-Pulse Inhibition to 112dB

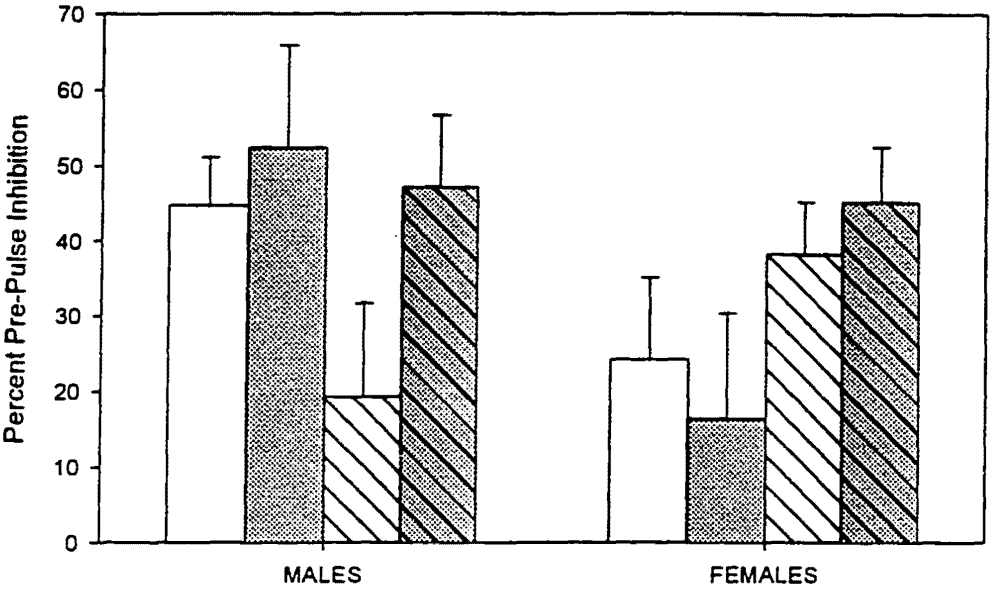
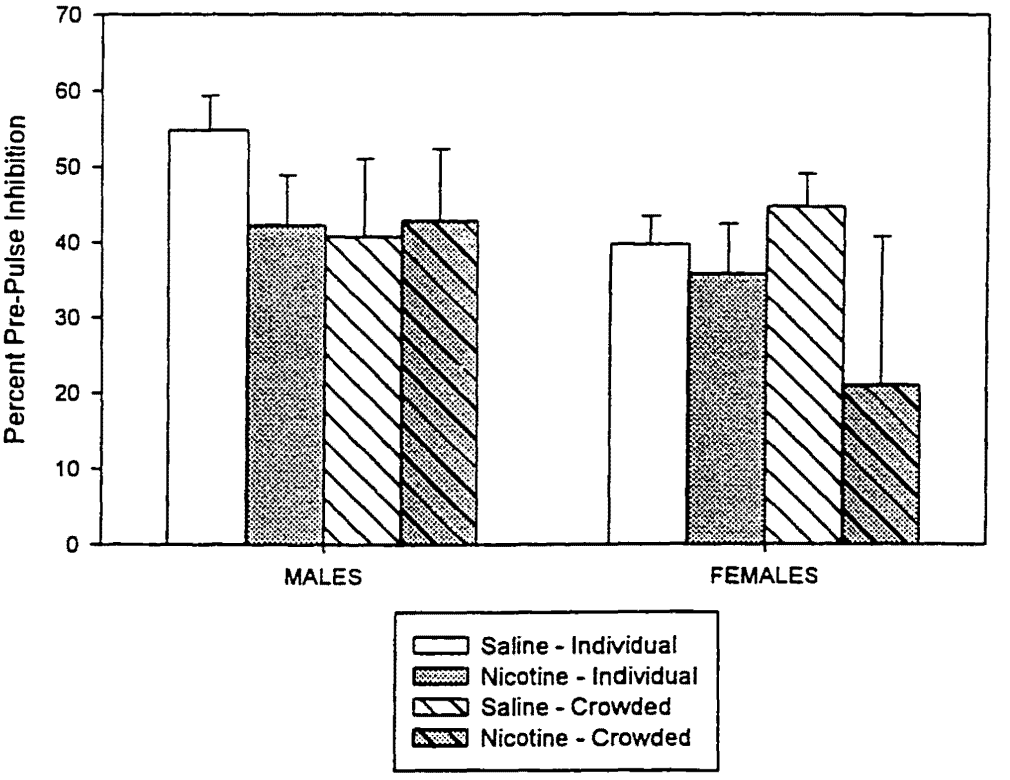


Figure 14: Cessation Day 3 Percent Pre-Pulse Inhibition to 122dB



Experiment 2: Body Weight

Figure 15a:
Long-Evans Males

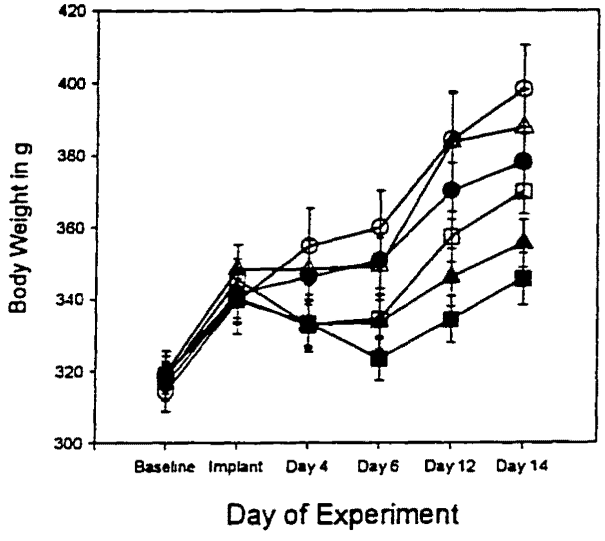


Figure 15b:
Long-Evans Females

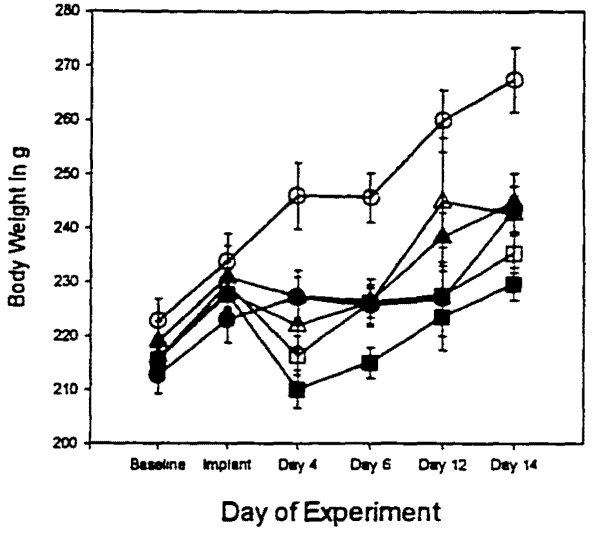


Figure 15c:
Sprague-Dawley Males

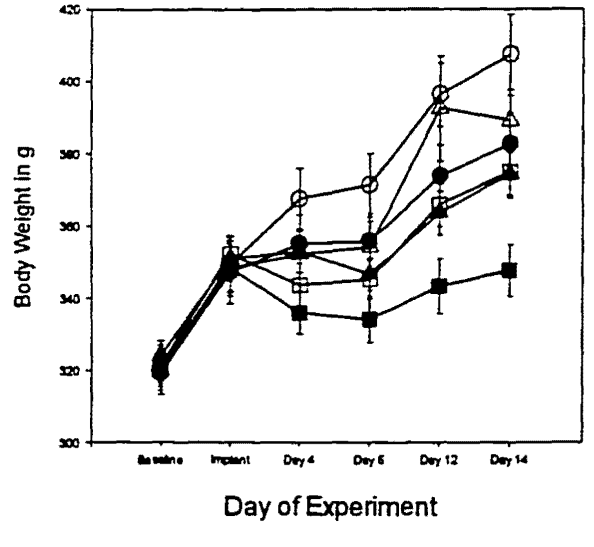
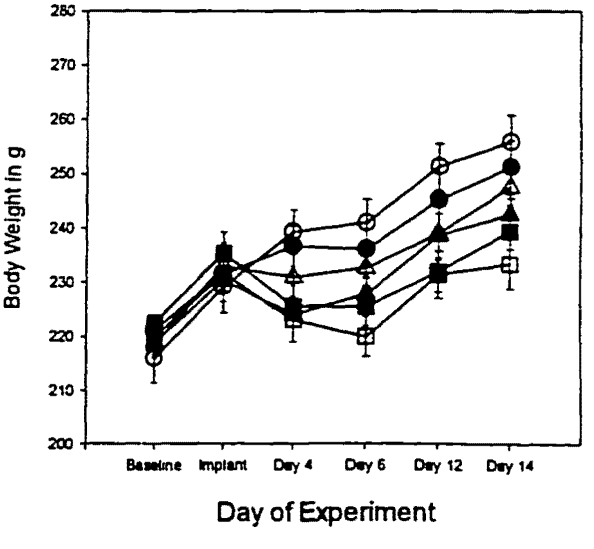


Figure 15d:
Sprague-Dawley Females



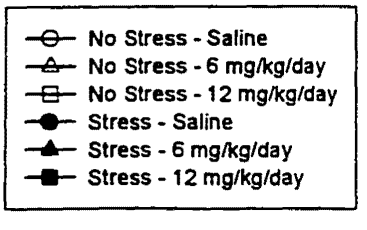


Figure 16a: Day 2 Startle Amplitude to 98dB Stimulus

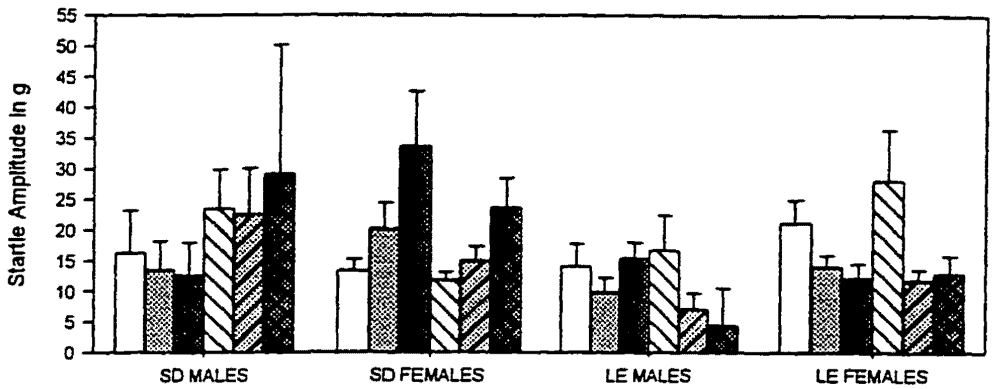


Figure 16b: Day 6 Startle Amplitude to 98dB Stimulus

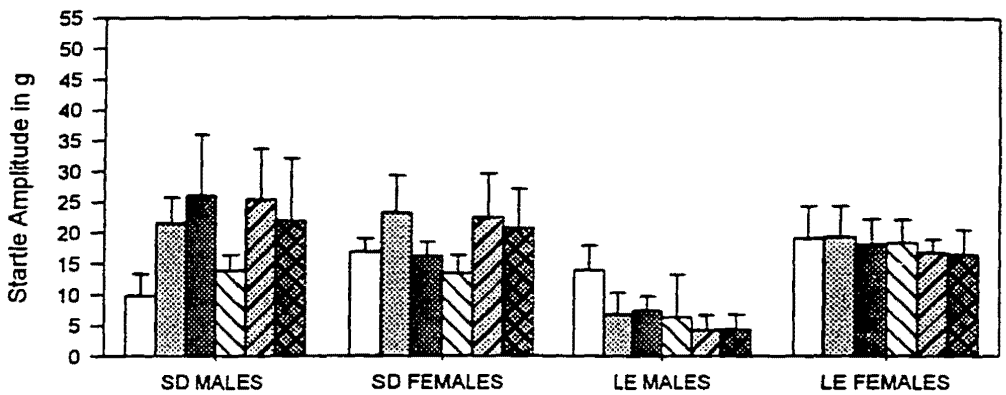
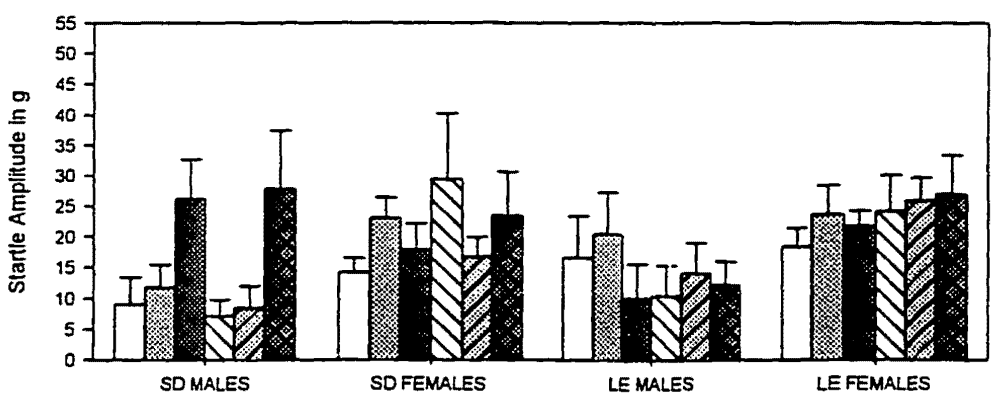


Figure 16c: Day 12 Startle Amplitude to 98dB Stimulus



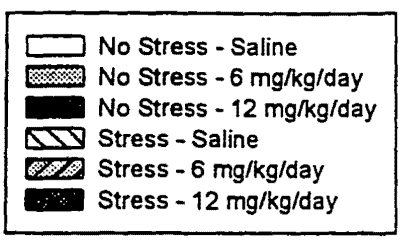


Figure 17a: Day 2 Startle Amplitude to 112dB Stimulus

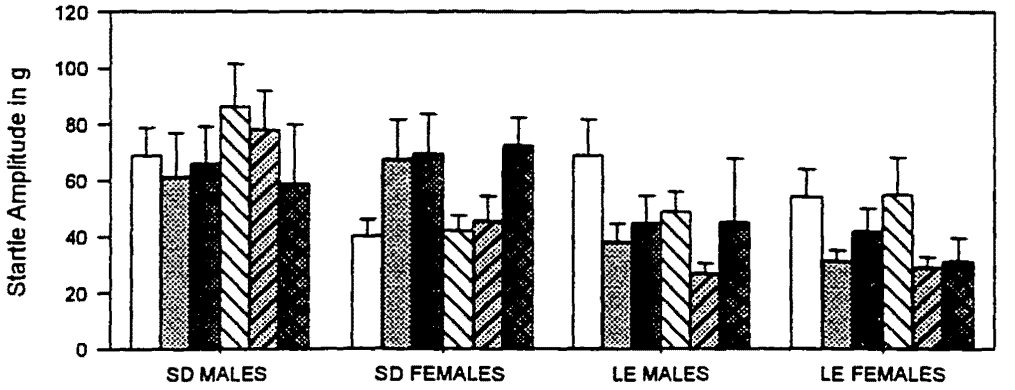


Figure 17b: Day 6 Startle Amplitude to 112dB Stimulus

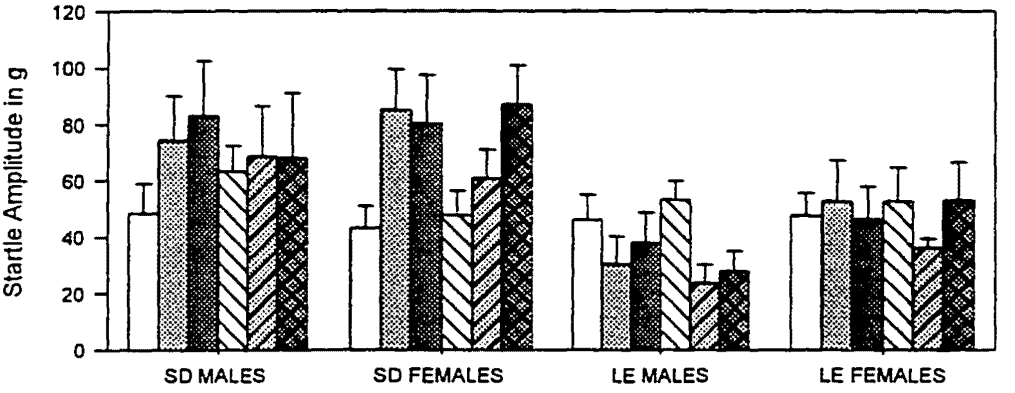
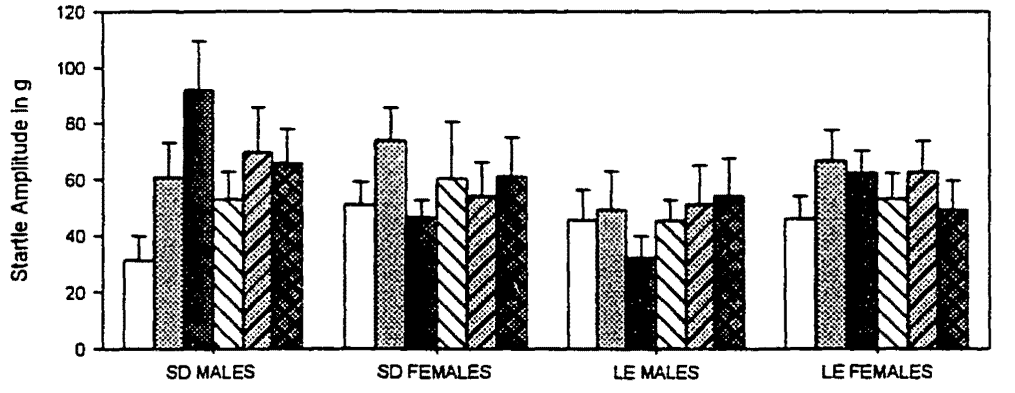


Figure 17c: Day 12 Startle Amplitude to 112dB Stimulus



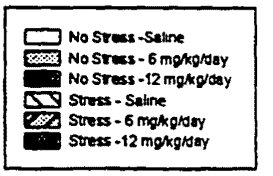


Figure 18a: Day 2 Startle Amplitude to 122dB Stimulus

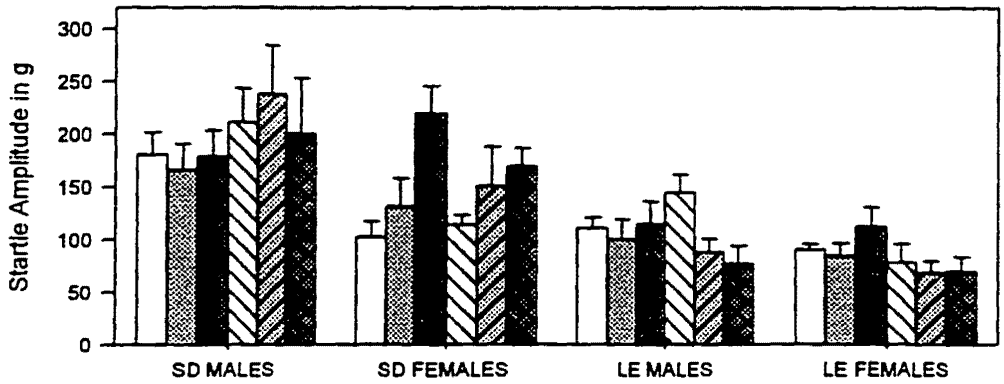


Figure 18b: Day 6 Startle Amplitude to 122dB Stimulus

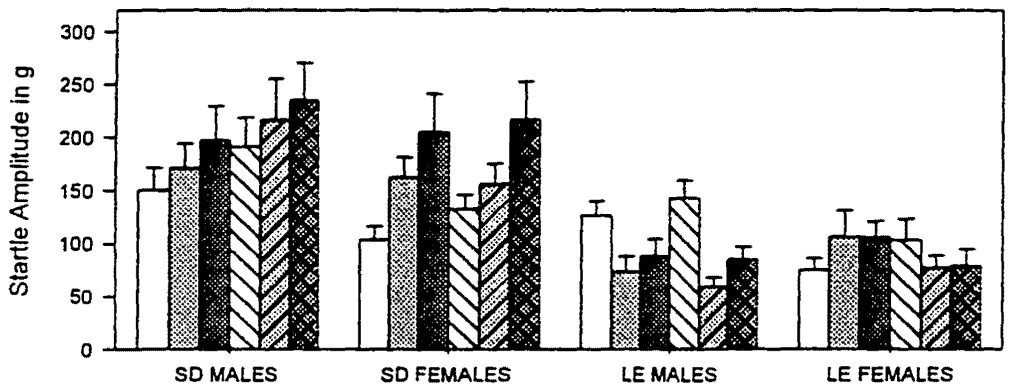


Figure 18c: Day 12 Startle Amplitude to 122dB Stimulus

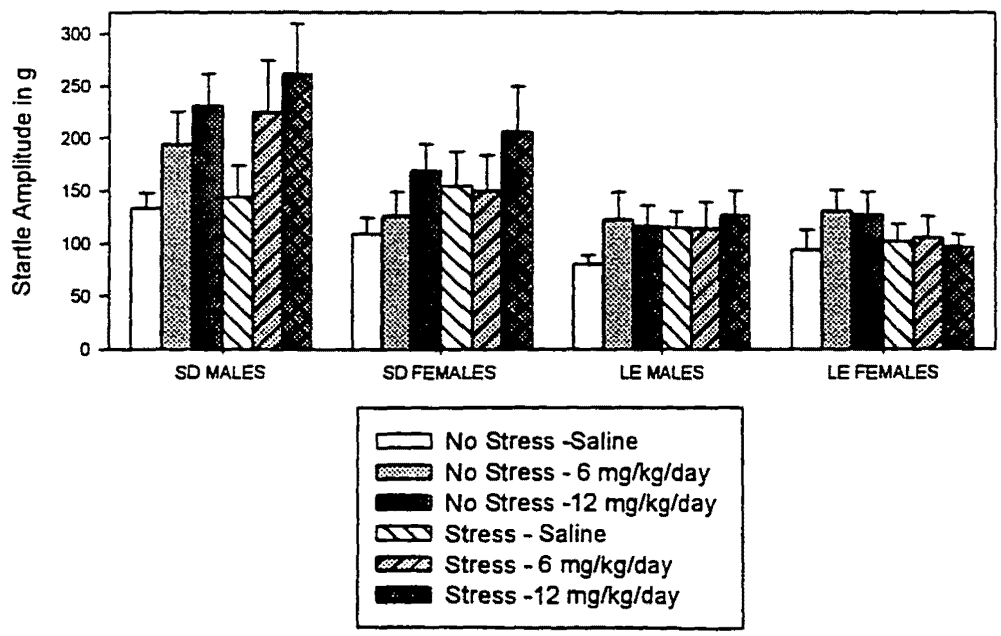


Figure 19a: Day 2 Pre-Pulse Inhibition Amount to 98dB Stimulus

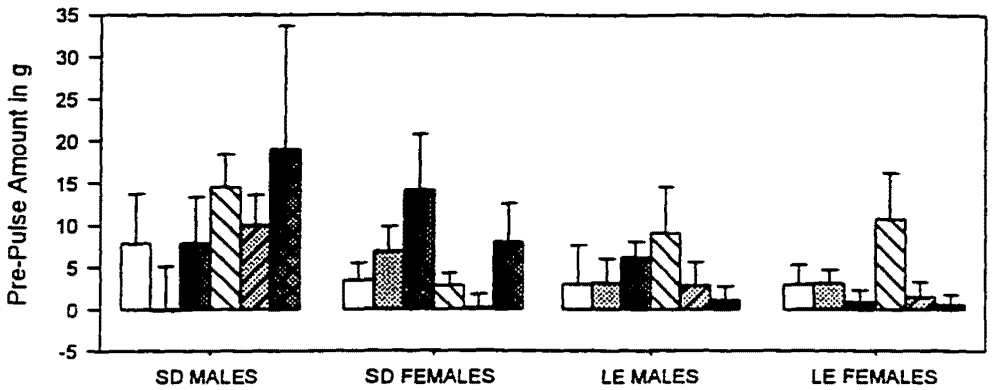


Figure 19b: Day 6 Pre-Pulse Inhibition Amount to 98dB Stimulus

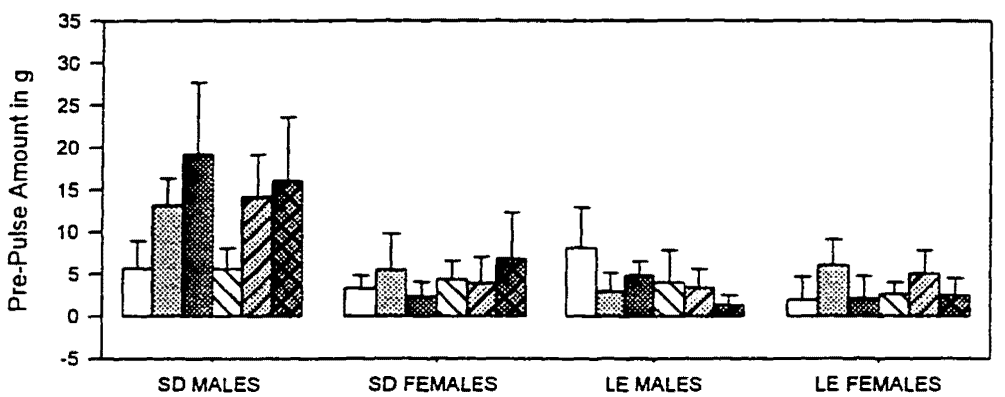
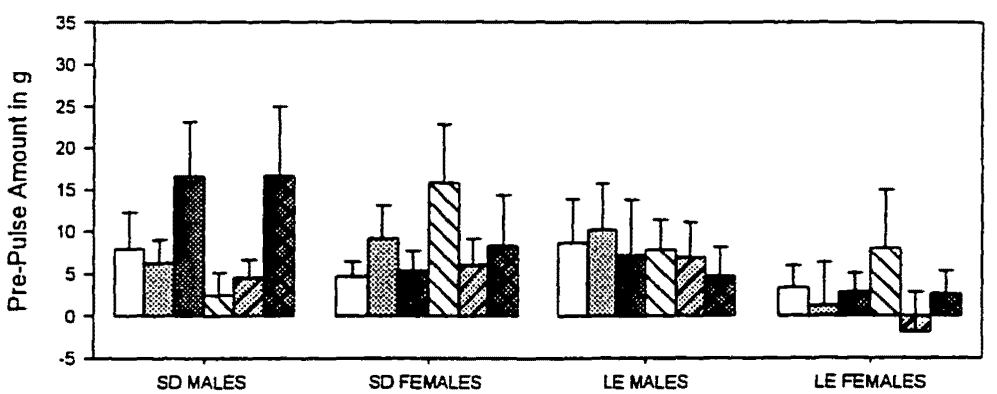


Figure 19c: Day 12 Pre-Pulse Inhibition Amount to 98dB Stimulus



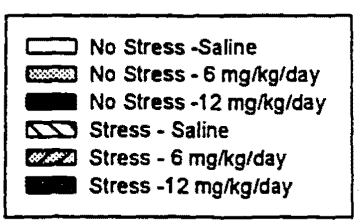


Figure 20a: Day 2 Pre-Pulse Inhibition Amount to 112dB

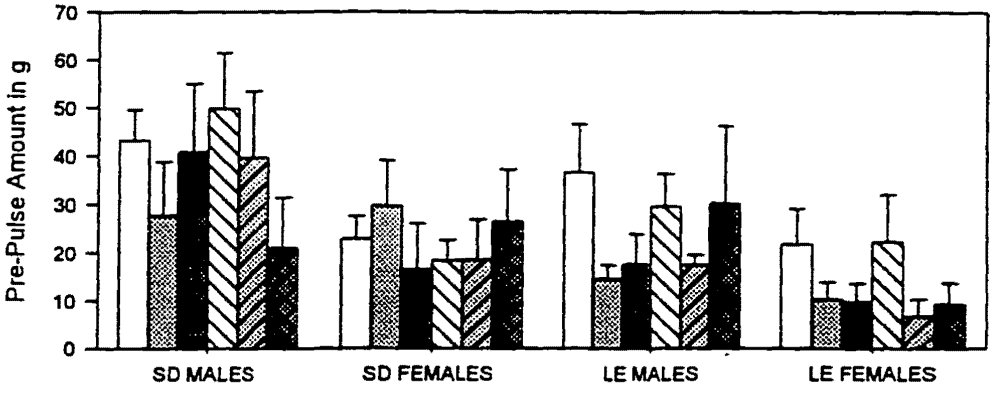


Figure 20b: Day 6 Pre-Pulse Inhibition Amount to 112dB

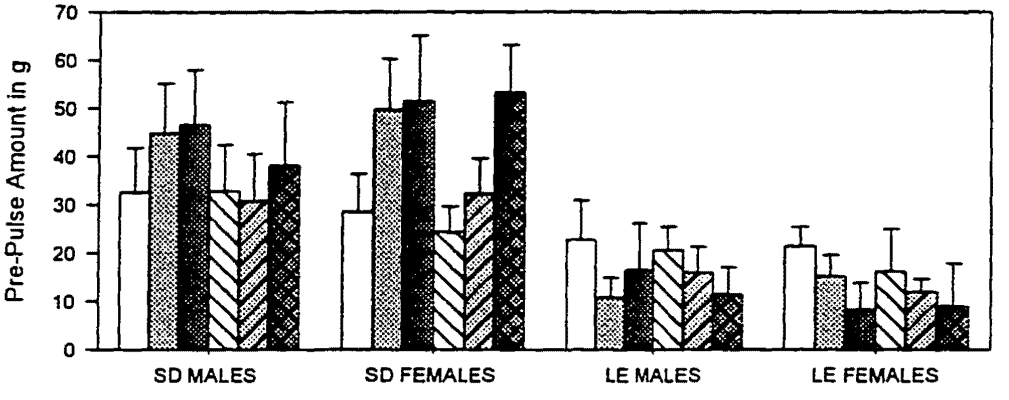
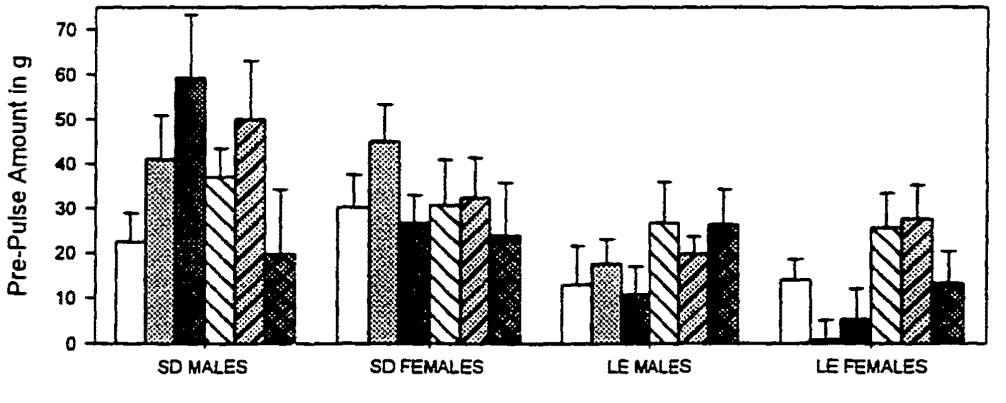


Figure 20c: Day 12 Pre-Pulse Inhibition Amount to 112dB



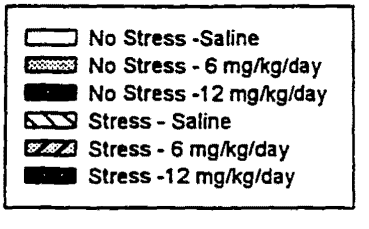


Figure 21a: Day 2 Pre-Pulse Inhibition Amount to 122dB

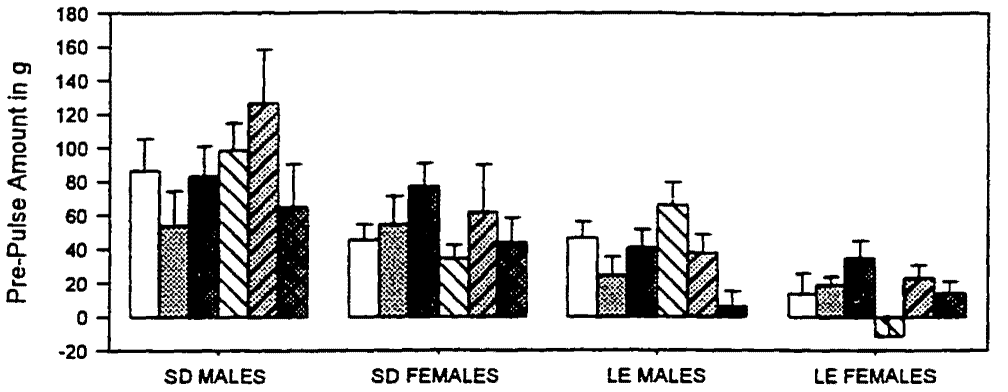


Figure 21b: Day 6 Pre-Pulse Inhibition Amount to 122dB

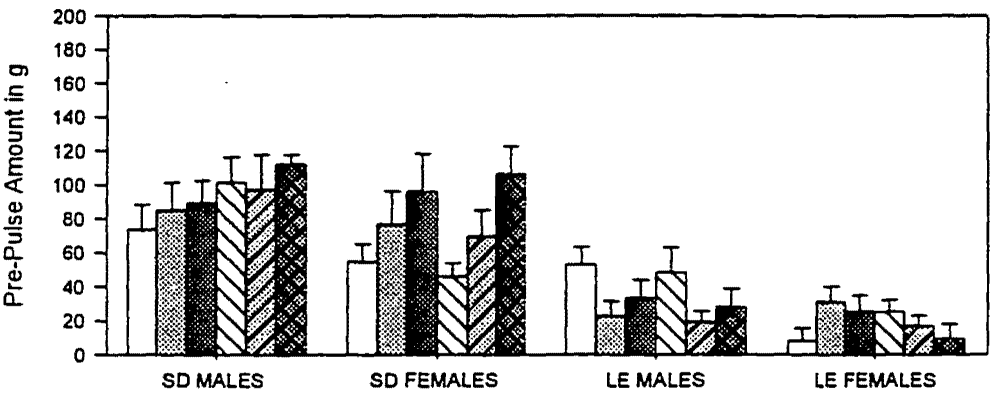


Figure 21c: Day 12 Pre-Pulse Inhibition Amount to 122dB

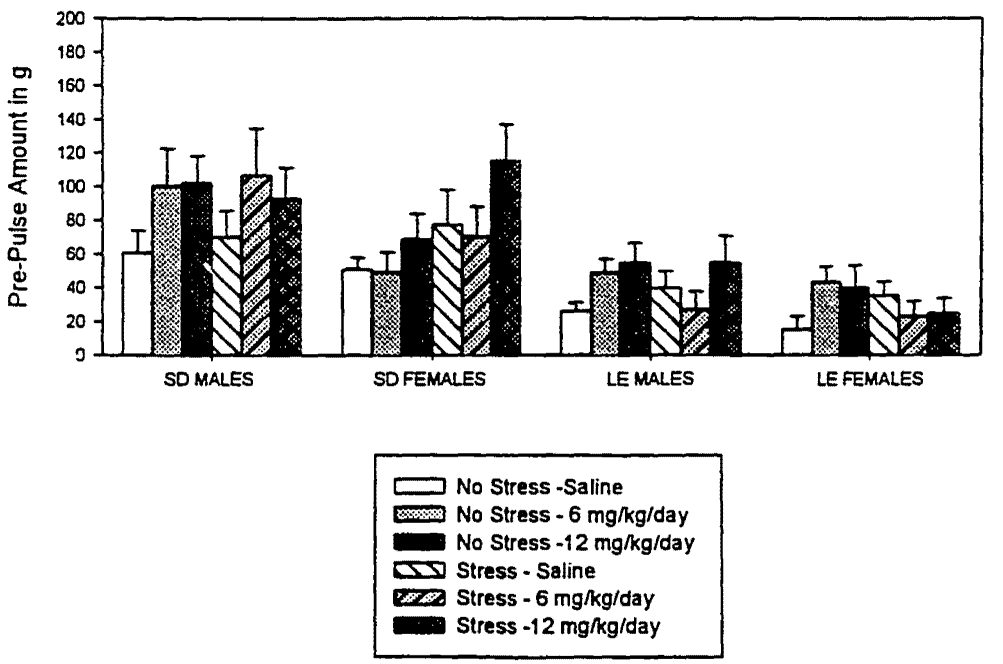


Figure 22a: Day 2 Percent Pre-Pulse Inhibition to 98dB

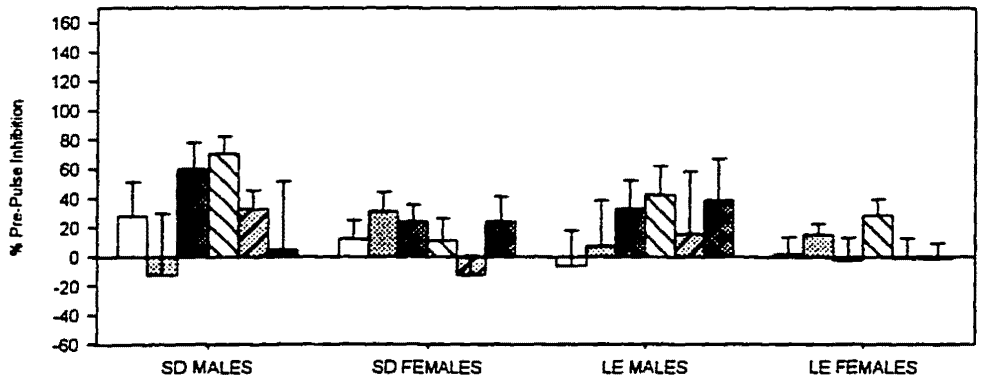


Figure 22b: Day 6 Percent Pre-Pulse Inhibition to 98dB

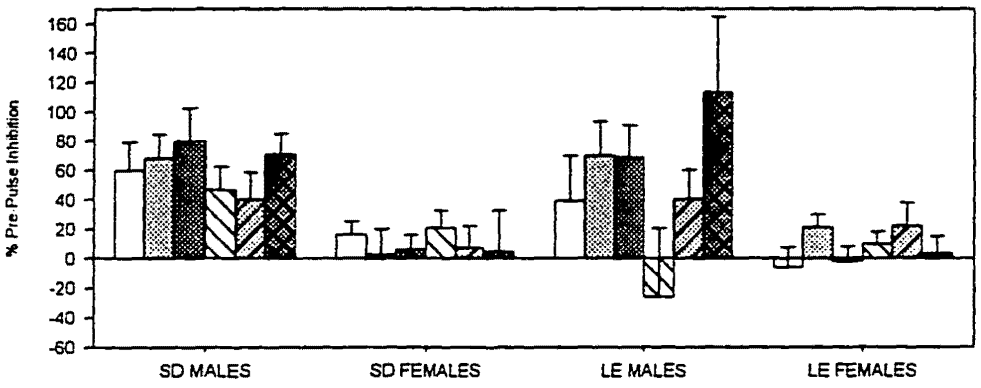
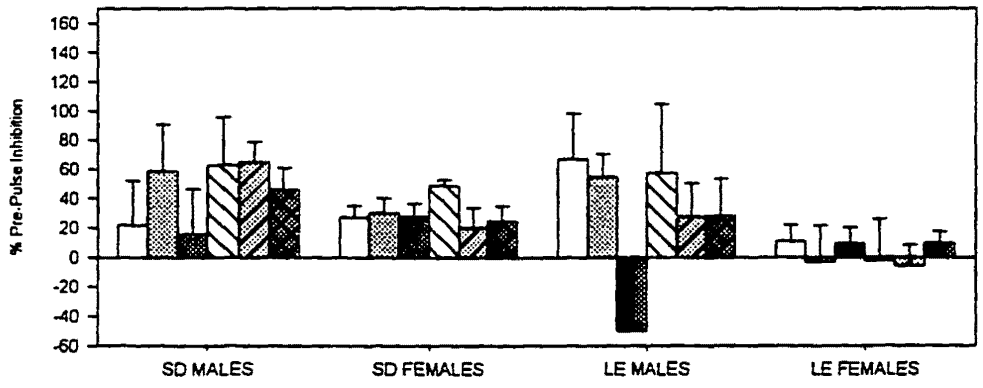


Figure 22c: Day 12 Percent Pre-Pulse Inhibition to 98dB



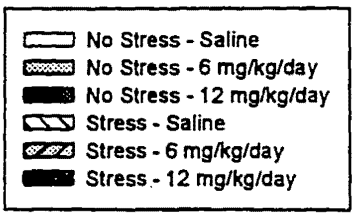


Figure 23a: Day 2 Percent Pre-Pulse Inhibition to 112dB

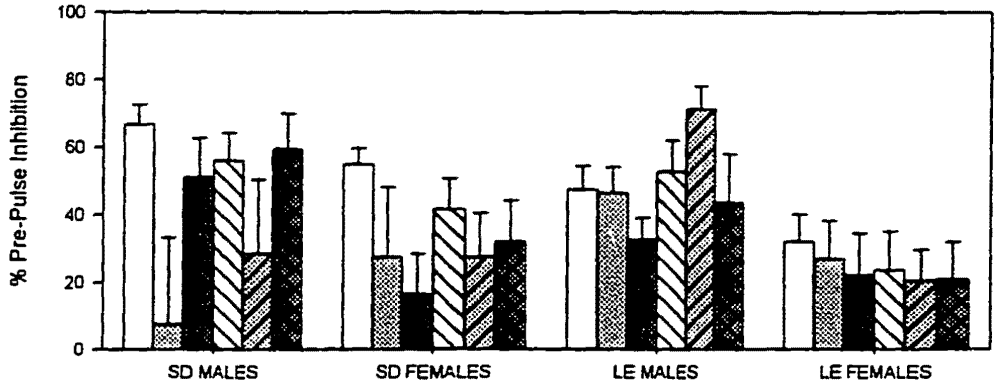


Figure 23b: Day 6 Percent Pre-Pulse Inhibition to 112dB

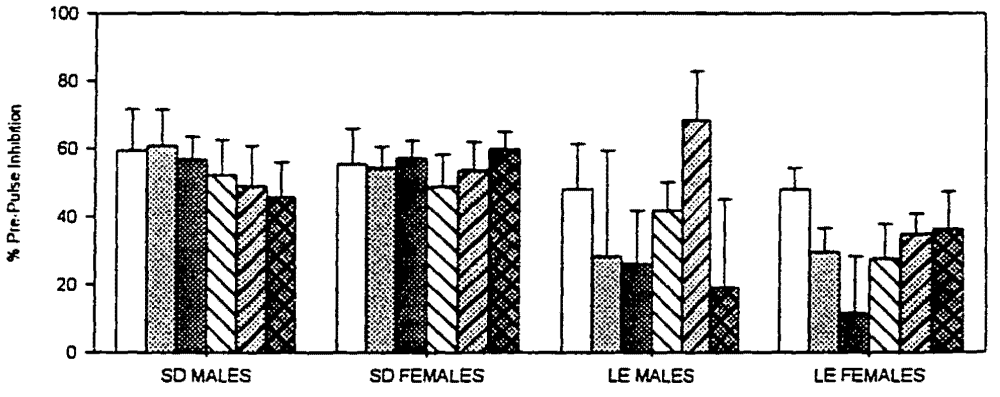


Figure 23c: Day 12 Percent Pre-Pulse Inhibition to 112dB

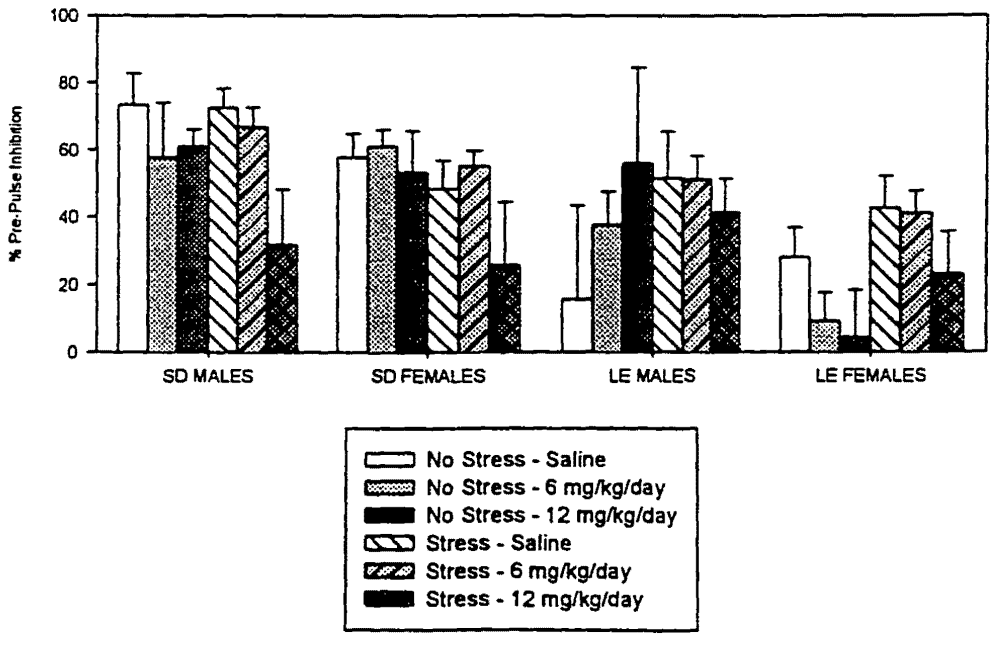


Figure 24a: Day 2 Percent Pre-Pulse Inhibition to 122dB

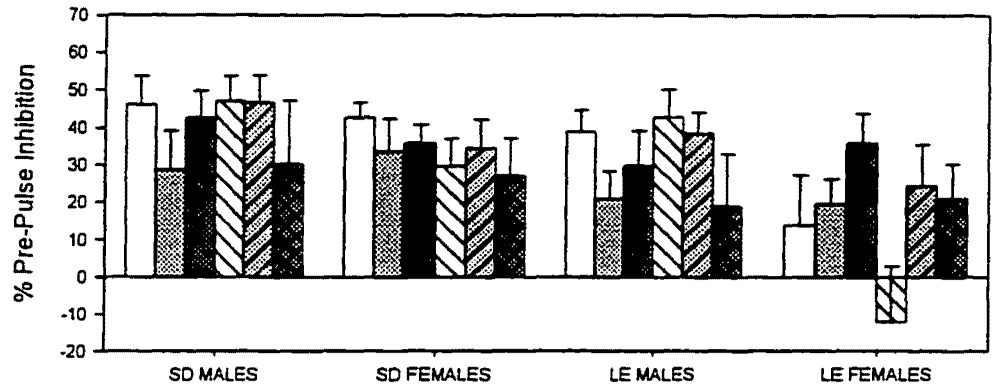


Figure 24b: Day 6 Percent Pre-Pulse Inhibition to 122dB

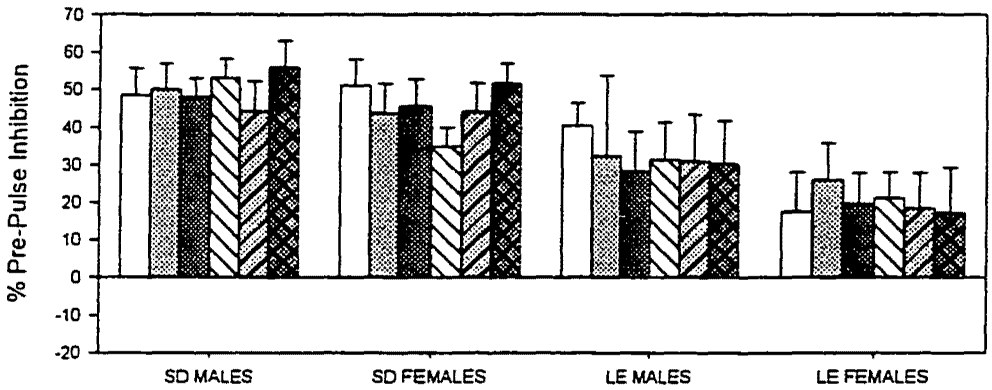


Figure 24c: Day 12 Percent Pre-Pulse Inhibition to 122dB

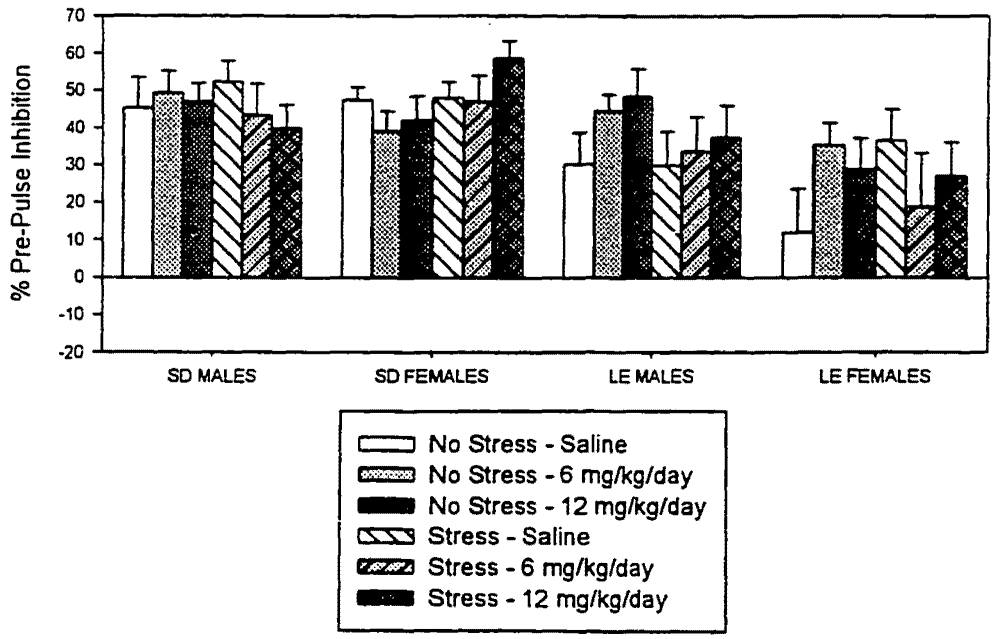


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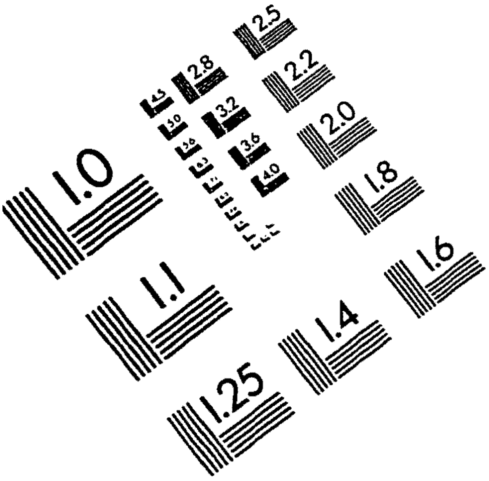
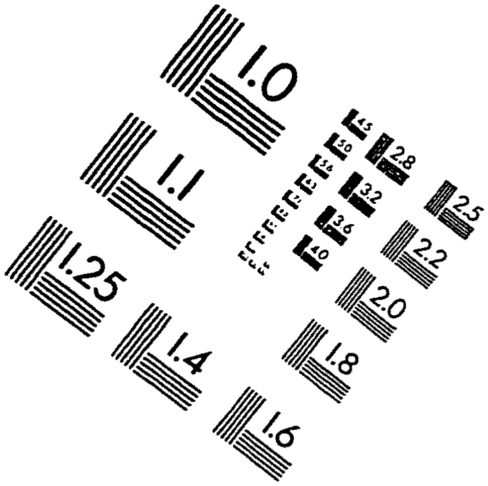
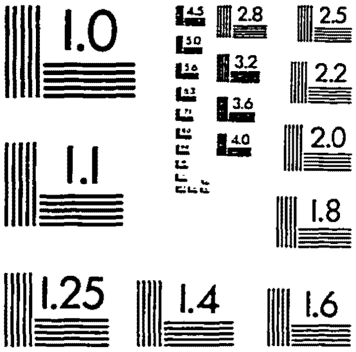
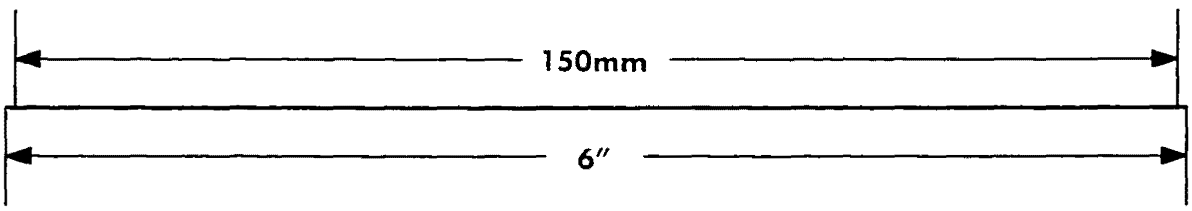
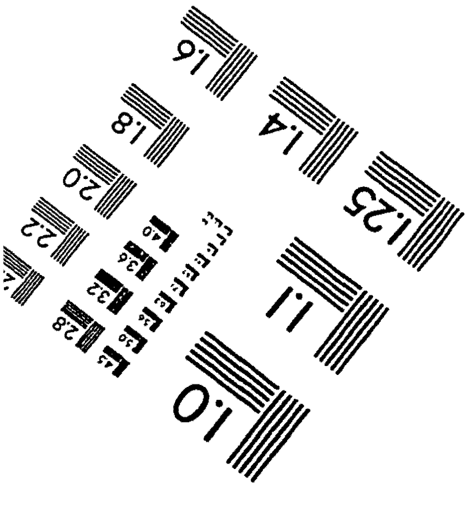
IMAGE EVALUATION TEST TARGET (QA-3)

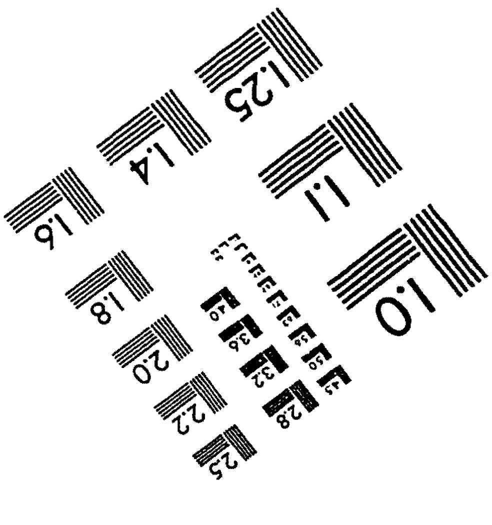












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